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Seasonal and Salinity Effects on the Distribution of Higher Filamentous Marine Fungi at Rookery Bay, FL.

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Abstract

More than 500 species of higher marine fungi in over 300 genera have been described. Many marine fungi are highly specialized for marine environments relative to their terrestrial counterparts, having appendaged ascospores and conidia to aid in buoyancy, entrapment, and adherence to substrates. They have been reported to inhabit a wide variety of substrates including decaying wood, leaves, calcareous and chitinous substrates, seaweeds, and seagrasses. Most early studies on marine fungi were carried out in temperate regions. Investigations have now shifted to tropical locations in order to better evaluate the abundance and diversity of marine fungi on a global basis. Many surveys have focused on mangrove habitats in the Pacific and Atlantic Oceans, resulting in the discovery of many new taxa. The purpose of this study was to examine the distribution and seasonal occurrence of higher marine fungi along a salinity gradient in a marine estuary, Henderson Creek in Rookery Bay Reserve Naples, Florida. Parameters including temperature and salinity were measured.

Three stations were established along Henderson Creek. Mean salinity ranged from 5 ppt at the low salinity station Visitor Center to 36ppt at the high salinity station Field Station. Substrates used for fungal collections were wood panels of a hardwood Oak (*Quercus* sp.) and a softwood Pine (*Pinus* sp.). Four panels were submerged at each station and removed in 3 months increments over the course of one year.

One-hundred-and-sixteen species of filamentous higher marine fungi were identified over the course of this study, including seventy-one Ascomycetes, three Basidiomycetes, and forty-one Deuteromycetes. There was no clear pattern of seasonality in the species composition. Total species diversity and richness decreased in each 3month period following the first 3 month period. Changes in salinity appeared to alter the ratio of Ascomycetes to Fungi Imperfecti

observed at each station. Marine fungi in this collection were compared with previous reports on the east coast of Florida (Adams, 2003a; Kukich, 2005; Vogel, Schatz, Laubach, & Rogerson, 2008). A higher total species number as well as greater diversity was observed in this study when compared with reports from mangroves in southeast Florida.

Marine fungi are active decomposers in mangrove environments and contribute to total dissolved organic matter in estuarine and near shore ecosystems. While most studies focused on the taxonomy of marine fungi, few have looked at their ecology. Further studies will have to be conducted to better determine the role of filamentous marine fungi in near shore and estuarine environments.

1. Introduction

Marine fungi are a worldwide ecological group distinct in their geographical distribution and substrata. Marine fungi have been heavily studied in temperate areas (Hughes, 1974; J. Kohlmeyer & Kohlmeyer, 1979; Petersen & Koch, 1997) and tropical mangroves (Alias & Jones, 2000; J. Kohlmeyer, 1980a, 1984; Schmit & Shearer, 2003) but less so in tropical and subtropical regions.

Marine fungi are heterotrophic eukaryotes classified as either obligate marine parasites on plants and animals, symbionts in lichenoid associations with algae, or saprobes on dead plant tissue (cellulose and lignin) and animal tissues (keratin and chitin) (Bugni & Ireland, 2004; Dighton, White, & Oudemans, 2005). By decomposing dead tissue marine fungi contribute to the total primary production (TPP) of ecosystems. Research emphasizing the contributions of marine fungi to the TPP has lead to further investigations of their distribution and occurrence in marine environments. Studies conducted in relation to the occurrence and distribution of filamentous marine fungi in the Atlantic, Pacific, and Indian Oceans (Jones & Puglisi, 2006; Sundari, Vikineswary, Yusoff, & Jones, 1996) have demonstrated their worldwide ubiquity and have allowed for a better understanding of the multiple roles of marine fungi.

Higher filamentous fungal research has largely focused on the effects of salinity, seasonal temperature, and substrate preference in a variety of habitats including mangrove forests (Sadaba, 1996) (dela Cruz, Wagner, & Schulz, 2006; Jones & Puglisi, 2006; Palmero Llamas, de Cara Gonzalez, Gonzalez, Ruiz Lopez, & Tello Marquina, 2008; Vogel et al., 2008).While there have been a number of studies on marine fungi in near-shore systems little is known of their seasonal distribution in South Florida and the effect changes in salinity might have on their occurrence. This study aims to examine the distribution, seasonal occurrence, and substrate

preference of higher marine fungi along Henderson Creek at the Rookery Bay National Estuarine Research Reserve in Naples, Florida.

1.1 Defining Marine Fungi

Marine fungi occur in a diverse range of ecological niches making it difficult to create a definition that encompasses all facets of this ecological group of microbial organisms. They exist in nature as decomposers of wood and seaweed, parasites of algae, coral, and mangroves, and as saprophytes in marine sediment (Jennings, 1983).

Various published definitions are not taxonomically based, but rather, are based on the eco-physiological characteristics of marine fungi. Johnson & Sparrow (1961) and Tubaki (1968) defined marine fungi as a function of their ability to successfully grow and propagate at known salinities. In comparison, Meyers (1968) referred to the actual physiological components necessary for their growth as the basis for his definition of marine fungi.

The definition provided by Kohlmeyer & Kohlmeyer (1979) is the most widely accepted. Facultative marine fungi are defined as, “those from freshwater or terrestrial meilieus able to grow in marine environments”. Obligate marine fungi are defined as, “those that grow and sporulate exclusively in the marine or estuarine habitat”.

However, the original criteria selected to define marine fungi were too narrow and focused heavily on the organism’s ability to grow in culture. Definitions failed to recognize how the fungi grew in actual marine systems. It was observed that ascospores in some cases became dormant until favorable conditions arose to promote germination and growth. Thus, species grown *in vivo* could not be validated as a marine species until they were found to grow successfully in a marine environment. As a result the original definition of Kohlmeyer &

Kohlmeyer (1979) was modified to the ability to germinate and form a mycelium under natural marine conditions.

1.2 Ecological Role of Marine Fungi

Marine fungi act largely as decomposers of woody and herbaceous substrata, dead animals, and animal parts. Some species of marine fungi have shown to be the etiologic agent of diseases in marine plants and animals. Others have been recorded as being capable of forming symbiotic relationships with various organisms (D. Hyde et al., 1998).

Marine Fungi as Decomposers

The decomposition of organic substrates by fungi results in the release of nitrogen and phosphorus into the environment. A portion of the substrate compounds are incorporated into fungal biomass. Fungal mycelia may in turn be grazed upon by invertebrates, resulting in the release of trapped nutrients into the ecosystem (Boddy & Watkinson, 1995).

In addition to fungi, marine borers are also degraders of lingo-cellulosic substrates. However, marine borers are unable to withstand low oxygen levels found in compact sediments (Blanchette, Nilsson, Daniel, & Abad, 1990). Lignocellylolytic bacteria in the sediment also play a role in decomposition, but it has been suggested that fungi are more active in the decomposition process (Holt & Jones, 1983).

Three types of lignocellulosic degradation by terrestrial fungi are: *white rot*, defined as extensive enzymic and non-enzymic degradation of all wood components; *soft rot*, where extensive enzymic decay of cellulose and hemicellulose is associated with limited lignin degradation; and *brown rot*, in which a rapid cellulose and hemicellulose decay is the result of non-enzymic oxidation with limited lignin degradation (Eaton & Hale, 1993).

While there is significant colonization of marine fungi on lignocellulosic substrates there is little evidence that they actually break down this material. Mouzouras (1989) studied wood samples colonized by marine fungi with decay features suggesting soft rot. Many fungal strains demonstrate the ability to utilize cellulose as a nutrient source including *Corollospora maritima* and *Monodictys pelagica* (Rohrmann & Molitoris, 1992) and *Juella avicenniae, Lignincola laevis, Nia vibrissa,* and *Stagonospora* sp. (Pointing, Vrijmoed, & Jones, 1998). Various species of cellulolytic fungi are able to utilize cellulose as a nutrient source over a salinity range of 0-34%. In contrast, Raghukumar *et al's* study (1994) is the only report of hemicellulose degradation (defined as the ability of a fungi to degrade xylan). Eleven fungal strains were found capable of hemicellulose degradation which resulted in the release of reduced sugars via hydrolysis of the xylan substrate (Raghukumar et al., 1994).

Marine fungi also serve as decomposers in mangrove ecosystems. Lee (1995) documented the role of mangrove ecosystems in support of offshore biological production. Microbes including bacteria, eumycotic fungi, the chytrids, the chromistan (formerly known as the oomycetes), the labyrinthulids, and the hypochytrids breakdown the polymeric compounds into dissolved particulate organic matter which is then used at other trophic levels (Hawksworth, Kirk, Sutton, & Pegler, 1995; Lee, 1995).

Calculating Fungal Biomass

Recent work focusing on accurately measuring mycelial biomass and productivity has lead to a better understanding of the role of mycelial decomposers in marine ecosystems. Newell (1996) found that various dominant fungal groups are major decomposers of herbaceous material in mangroves and saltmarsh ecosystems. Bremer (1995) incubated decaying mangrove leaves

with or without nutrititive media (such as agar or corn meal) and found that the dominant fungi were non-marine species including many that are common in soils (e.g. *Cladosporium* spp., *Fusarium* spp., *Pestalotia* spp., and *Penicillium* spp.). Additionally, Bremer (1995) found a small number of obligate marine fungi including *Lanceispora amphibian* but at much lower frequencies. However, Singh and Steinke (1992) found *in vitro* celluloytic activity in samples of non-marine fungi from mangrove habitats suggesting they were responsible for herbaceous decomposition. It is still not completely clear which specific fungal group is responsible for the *in situ* decomposition of mangroves or if it is even possible to quantify fungal biomass in mangrove leaves *in situ* (D. Hyde et al., 1998).

Several attempts and varying methodologies have been applied to measure marine fungal biomass within decaying mangrove leaves (Blum, Mills, Zieman, & R.T, 1988; Newell, 1992). Blum *et al* (1988) and Newell (1992) detected low fungal biomass (<1 mg g^{-1} organic decaying-system mass) by means of direct microscope determinations of microbial abundance and cell volume. Blum *et al.* (1988) found that the bacterial biomass predominated through the decomposition process with fungi constituting only 0-20% of total microbial biomass.

Adams (2003) investigated the quantification of fungal biomass in selected substrates via ergosterol analysis techniques. ergosterol is a sterol component of the plasma membrane of eumycotic fungal cells (Potila, Wallander, & Sarjala, 2009). Ergosterol is unique to fungi and is not found in other eukaryotes. By quantifying ergosterol once can quantify fungal biomass. Adams (2003) measured the seasonal change in fungal biomass in intertidal and submerged wood substrates in a South Florida mangrove ecosystem. Mesh bags containing four different substrates (Red Oak, *Quercus rubra*, Yellow Pine, *Pinus leiophylla*, and Red Mangrove wood, *Rhizophora mangle*, and *R. mangle* leaves) were placed in subtidal and intertidal zones and

ergosterol levels were measured in January, March, May, and September. It was concluded that ergosterol levels were higher in late spring and early summer. It was suggested that higher water levels in the winter and fall increased competition for substrates resulting in lower numbers. Ergosterol levels were lowest in the submerged Red Mangrove leaves relative to the intertidal Red Mangrove leaves. Newell (1997) found similar results concluding that leaves in the upper intertidal zone exposed to periodic desiccation might favor eumycotic fungal growing conditions, whereas mycelial prostits called Oomycetes, may out compete higher filamentous fungi in submerged leaves. A survey of marine fungi along the Loxahatchee River showed a species composition that is similar to reports from other tropical and subtropical regions. Seventy-three percent of identified species were Ascomycetes, including *Marinosphaera mangrovei, Hypoxylon oceanicum, Cytospora rhizophorae, and Caryosporella rhizophorae* (Adams, 2003a)

1.3 Marine Fungal Symbioses

Terrestrial fungi exhibit several examples of symbiosis with insects such as mound building termites (*Macrotermes bellicosus*) and the mushroom *Termitomyces*, as well as ambrosia beetles(*Xylosandrus crassiusculus)* and the *Ambrosiella* fungus (Breznek, 2004). The relationships between fungi and marine organisms range from saprotrophic, symbiotic, and parasitic. Five distinct symbioses include holothurians, lichens, mycophycobioses, mycorrhize, and algicolous associations.

Saprotrophic marine fungi have been isolated from the surface, guts, and coelomic fluids of holothurians (Pivkin, 2000). These fungi displayed high proteolytic activity which has been suggested as a factor for fungal pathogenicity (St. Leger, Durrands, Charnley, & Cooper, 1988). The holothurians however have triterpene glycosides which have fungitoxic, hemotoxic, and

cytotoxic properties. The body of the most holothurians is comprised of 1.9%-9.0% protein (mainly collagen) (Levin, 1982). Fungi damage the holothurian tissue by means of proteolytic activity. In the study by Pivkin (2000) on a holothurian species it was shown that the fugnal diversity of fungi was greater on the exterior relative to the colemon. However, large amounts of *Cladosporium brevicompactum* and *Cladosporium sphaerospermum* were common in the holothurian coelom. To determine the pathogenicity of selected holothurian species, Pivkin (2000) looked at the gelatinolytic activity of by measuring the amount of denatured collagen. Fungal strains of both *Cladosporium brevicompactum* and *Cladosporium sphaerospermum* were twice as active in liquefying host protein relative to isolates found on the holothurian species surface (Pivkin, 2000).

Lichens are mutualistic associations between a fungus and an alga or cyanobacterium. The photosynthetically active alga is encased by the fungus which forms a structure called a thallus (Hawksworth, 2000). Once the thallus is formed the fungus undergoes a metabolic transformation, synthesizing and releasing various chemical compounds often referred to as lichen acids (Hill, 1994). There are roughly 15,000 species of lichen-forming fungi suggesting the high level of success of this particular symbiosis. Interestingly, there are only about 30 different types of algae and cyanobacteria that have been reported as the photosynthetic component.

Marine lichens often occur on intertidal coastal rocks because they are unable to survive constant subtidal immersion (Dighton et al., 2005). However there are some exceptions such as *Verrucaria serpuloides* which can grow subtidally as well as in the intertidal zone (Hawksworth, 2000). Various ecological factors affecting marine and maritime lichens have been discussed by Fletcher (1975). In general, it was observed that the duration and frequency of immersion in

addition to rock type are important factors contributing to the growth and development of marine lichens. Hawksworth (2000) reported that the most common lichen-forming algae in both freshwater and marine environments included, "…*Calothrix* (e.g. in *Lichina*), *Nostoc* (e.g. in *Pyrenocollema*), *Stichococcus* (e.g. in *Staurothele*), *Stigonema* (e.g. in *Ephebe*), and *Coccobotrys*, and *Dilabifilum*, and *Heterococcus* (all in *Verrucaria*)". Hawksworth (2000) also explored the dispersal of lichen reproductive units in the marine environment. He cited the specific example of *Staurothele* where small algae cells grow inside the fungal perithecium to be dispersed with ascospores. The ascospores of this genus are large and broadly ellipsoid suggesting an adaptation allowing spores to become lodged in cracks of rocks or carried off by grazing invertebrates.

Mycophycobioses refers to symbiotic associations between a marine fungus and a macroalga species (D. Hyde et al., 1998). In these particular relationships the algal partner predominates. The fungus grows between cells of the algal thallus without penetrating or damaging the host cells. The affect of each symbiont is still unclear; however it is thought that the fungus may protect the alga from drying out at low tide when it is exposed to ambient atmospheric conditions. It has been suggested that the fungus may also be required for algal growth and development. Garbary and London (1995) found that spores of marine algae, such as *Ascophyllum nodosum,* fail to develop a thallus unless infected by its symbiotic fungus.

Common fungal genera with mycophycobiotic associations include *Blodgettia* and *Mycosphaerella. Blodgettia bornetii* is a hyphomycete which grows in the tropical algal species *Cladophora*. "The fungus produces spores and is absent only from the growing tips of the alga" (Paracer & Ahmadjian, 2000). *Mycosphaerella ascophylli* is associated with the brown algae *Ascophyllum nodosum* and *Pelvetia canaliculata*. "Hyphae grow between the cells of the host,

and the fungus undergoes sexual reproduction and produces fruiting bodies while still within the host" (Paracer & Ahmadjian, 2000).

Turgidosculum complicatum displays a unique mycophycobiotic association with the green algae *Praseola borealis* and *P. tessellata*. Hyphae of the fungus grow throughout the algal thallus, separating the cells into groups of four or into rows. Unlike other mycophycobiotic associations, the alga is able to live without the fungus. Species of *Prasiola* thalli growing with the fungus are very common in exposed or drier parts of the intertidal zone, suggesting that the fungus protects it in these harsh conditions. (J. Kohlmeyer & Kohlmeyer, 1979).

Additional marine fungal species occur in associations with seaweeds. Algal-inhabiting fungi are referred to as algicolous. They are a taxonomically diverse group of mutualists, including endosymbionts, parasites, pathogens, and saprobes. Many algicolous fungal genera are exclusively associated with algal species including *Spathulospora, Chadefaudia, Haloguignardia, Retrostium, Hisidicarpomyces,* and *Pontogenia* (Dighton et al., 2005).

Schatz (1984) studied the occurrence of the ascomycete *Didmosphaeria danica* infecting the economically important alga *Chondrus crispus*. The study resulted in the description of a new genus *Lautitia* with *Lautitia danica* (Berlese) comb. nov. as the type species. *Lautitia danica* specifically infects the reproductive tissues of susceptible host plants. Infections are first indicated by the appearance of spermogonia on cystocarpic and tetrasporic plants. Schatz (1984) found that there was a higher susceptibility of cystocarpic plants to infection when compared with tetrasporic plants, possibly due to differences in carageenin type. It was also observed that the life cycle of the parasite is dependant to some degree on the ambient water temperature. The time course of the life cycle decreased with increasing water temperatures in the summer months. Based on sporadic collections made from drift material rather than periodic sampling of

living hosts over a full season it was found that only sublittoral *Chondrus crispus* was found to be infected. Prior studies suggested that much higher proportions of reproductive populations were infected by *L. danica* (Wilson & Knoyle, 1961).

Schatz (1984) also investigated the infection of a brown alga, *Laminaria saccharina* by *Phycomelaina laminariae* and subsequent degredation by saprobic fungi. Healthy and infected attached plants of *L. saccharina* were placed in mesh bags, suspended in the water column, and examined at three-month intervals for nine months. The number of fungal species isolated was higher in the stipe tissue infected by *P.* laminariae compared to uninfected stipe tissues. *Acremonium incoloratum* was a common isolate from infected attached stipes and from detached healthy stipes submerged for six months. These findings suggested that this particular species is an opportunistic colonizer of senescing or dead plant tissue. Additionally *Bartalinia robillardoides* was identified, its first ever recorded occurrence from a marine substratum in North America. A total eighteen fungi were identified on the stipe tissue of *L. saccharina* including two true marine fungi *Dendryphiella salina* (the most common fungal invader of the macro-algae) and *Zalerion maritimum* (Schatz, 1984). Carbon levels of detached, suspended, infected tissues increased in the first six months while stipes of healthy tissues decreased only increased during the second three-month period. The loss of nutrients was attributed to high competition rates lowering available organic nutrients on the substrate and feeding in particular by nematodes, sea worms (*Nereis*), various larvae, and other invertebrates as water temperatures increased (Schatz, 1984).

Schatz (1984) did not support the findings of Chester and Bull (1963) that marine fungi are not an active component of the microbiota involved in the utilization of detrital brown algae because of their inability to degrade laminarin. However, the primary storage product of

Lainaria is mannitol (Haug & Jensen, 1954). *Dendryphiella salina*, which is capable of using laminaria as a carbon source (Tubaki, 1969) was regularly found on infected and healthy stipe tissues throughout the study. *Dendryphiella salina* is also able to degrade alginates, a component in the cell walls of brown algae (Wainwright, 1980). Consequentially this enzymic capacity in addition to the invasive nature of fungal growth studied by Schatz (1984) contrasted with the views of Chester and Bull (1963), implicating *D. salina* as a major decomposer of brown algal sporophytes in temperate waters.

1.4 Taxonomy, Biodiversity, and Phylogeny of Marine Fungi

Fungi, Viridiplante, and Animalia are three of the largest clades, hypothesized to have descended from unicellular, flagellated, aquatic forms that were able to disperse on land (James, Kauff, Schoch, Matheny, Hofstetter, Cox, Celio, Gueidan, Fraker, Miadlikowska, Lumbsch, Rauhut, Reeb, Arnold, Amtoft, Stajich, Hosaka, Sung, Johnson, O/'Rourke et al., 2006). A number of biologists have developed hypotheses regarding evolutionary features of morphology and ecology of both plants and animals. However, no such phylogenic hypothesis has been developed to explain the ancestral form and nutritional mode of early fungi (James, Kauff, Schoch, Matheny, Hofstetter, Cox, Celio, Gueidan, Fraker, Miadlikowska, Lumbsch, Rauhut, Reeb, Arnold, Amtoft, Stajich, Hosaka, Sung, Johnson, O'Rourke et al., 2006). Pironzynski and Malloch (1975) suggested that the shift from an aquatic to a terrestrial existence was driven by the development of mycorrhizal symbioses involving fungal hyphe and plant roots.

It is currently thought that fungi with flagellated cells (Chytridiomycota) are a sister group to phyla of non-flagellated fungi (Zygomycotam Glomeromycota, Ascomycota, and Basidiomycota) suggesting that the loss of the single flagellum propagated a shift to land (James,

Kauff, Schoch, Matheny, Hofstetter, Cox, Celio, Gueidan, Fraker, Miadlikowska, Lumbsch, Rauhut, Reeb, Arnold, Amtoft, Stajich, Hosaka, Sung, Johnson, O/'Rourke et al., 2006) Studies have shown that some marine fungi vary from their terrestrial and freshwater relatives taxonomically, morphologically, and physiologically (Barghoorn ES & DH, 1944; Johnston & Sparrow, 1961; J. Kohlmeyer & Kohlmeyer, 1979). Many marine fungi however occur in multiple habitats i.e. terrestrial and marine. This is demonstrated by *Savoryella lignicola*, and *Lignincola leavis* which have been observed in marine as well as freshwater habitats. Similarly, species of *Leptosphaeria, Pleospora, Trematosphaeria* ,*Calathella* and *Alternaria* are also found in both aquatic and terrestrial habitats.

Based on their original survey of marine fungi in 1979, Kohlmeyer and Kohlmeyer concluded that there were less than 500 species of marine fungi and thought that, "considerable additions of new species in the future [were] unlikely". Since that time however, several new species have been described. At present there are approximately 1500 species of marine fungi (Heip, 2007).

1.5 Substrate Preference of Marine Fungi

Fungal biodiversity and distribution is largely determined by the available substrata. The marine environment provides an array of substrata for fungi to colonize. The most common substrates are drift wood and mangroves and their associated mycobiota, such as fallen leaves and wood, and submerged roots. Other substrata include sediments, algae, coral, calcareous tubes of mollusks, intertidal grasses, and living animals as well as the guts of crustaceans (D. Hyde et al., 1998). Free floating wood in the ocean favors the growth of the members of *Haloshpaeriales* which are characterized by appendaged ascospores that aid in flotation and attachment (Jones, 1994).

Marine fungi are adapted for the attachment to and colonization of solid substrates (Dighton et al., 2005). Compositional changes in the substrate affect the species composition rather than the biodiversity (Dighton et al., 2005). Members of the order Halosphaeriales are commonly found on submerged timber, while members of the subclass Loculoascomycetes are more common on intertidal mangrove wood (Jones & Alias, 1997). While marine fungi can colonize an array of substrates, over 90% of higher marine fungi utilize woody or herbaceous substrates (Dighton et al., 2005). Hyde (1990) concluded that lignocellulosic materials support the highest level of species diversity. By contrast only a few species are able to colonize calcareous materials or sand grains (K. D. Hyde, 1990b).

Kirk and Brandt (1980) investigated the seasonal substrate preference of marine fungi in the lower Chesapeake Bay. Panels of kiln dried pine and birch wood were chosen in this study of substrate preference. Over a one year period, 23 ascomycetes, 14 deuteromycetes, and 27 marine taxa representing facultative and terrestrial marine species were identified. The total number of species present increased from winter to fall, with the most significant increase in species diversity occurring in the spring (Kirk & Brandt, 1980). Preference of a substrate was characterized by a large production of hyphae and fruiting structures on incubated or freshly extracted panels. Seven of the species showed a strong preference for the birch wood as a substrate. However, no single species occurred exclusively on the pine or birch panels. Fresh panels submerged for 3 months had vast colonies of *Halosphaeria appendiculata, Halosphaeria mediosetigera, Halosphaeria quadriremis, Leptosphaeria oraemaris,* and *Cirrenalia macrocephala*. In contrast, incubated panels revealed a greater abundance of *Ceriosporopsis calyptrate, Lignincola laevis,* and *Microthelia linderi*.

Hyde (1990) studied the substrate preference of marine fungi in mangrove ecosystems at Kampong Kapl and Tungit Api Api in Brunei. The mycota of five mangrove tree species (*Avicennia alba, Rhizophora apiculata, Rhizophora mucronata, Sonneratia alba, and Xylocarpus granatum)* in the intertidal zone were investigated. The dominant fungal communities differed across the five host mangrove species. Seventy-five species of Ascomyetes and one species of Basidiomycetes exhibited a strong host preference, e.g *Caryospora mangrovei,* and *Aigialus mangrovis.* Other species showed no preference, developing on multiple tree species, e.g., *Hypoxylon oceanicum, Leptosphaeria australiensis* and *Savoryella lignicola* (K. D. Hyde, 1990a).

1.6 Marine Fungi and Mangroves

Photosynthetic phytoplankton produce organic matter, part of which is secreted directly into adjacent waters. Similarly protistan and mesozooplankton grazers release organic matter when undigested remains are emptied from food vacuoles. These excreted materials are termed together as “dissolved organic matter” or DOM. Dissolved organic matter is a major nutrient resource for heterotrophic and pelagic bacteria. Heterotrophic pelagic bacteria are in turn consumed by prostists (mostly nanoflagellates, and parasitized by viruses). Nanoflagellates are preyed upon by larger protozoa and in some cases larger mesozooplankton (e.g. nauplii) or mucoid filter feeders (e.g. appendicularians).This sequence of organic matter to dissolved organic matter is a unique sequence in marine food webs and is referred to as the microbial loop (C. Miller, 2004).

Mangroves are crucial components of tropical and subtropical food webs. Mangroves occur in worldwide in 112 countries, covering 10-24 million hectares with a global biomass of

8.7 Gton dry weight and 4.0 Gton of carbon (Twilley, Chen, & Hargis, 1992). The woody tissue of mangroves accounts for 20-50% of the total net primary production of a mangrove forest, also referred to as a mangal (Hogarth, 1999). Marine fungi play a major role in the mangal assisting in the decomposition process of woody and non-woody plant material. Mangrove inhabiting fungi were first described from mangroves in Australia by Cribb and Cribb (1955). Due to the high occurrence of exogenous nitrogen in the mangal, fungi are able to convert plant carbon compounds, such as lignin and cellulose, into microbial protein(Newell, Fell, Statzellitallman, Miller, & Cefalu, 1984). That protein in turn is utilized as a major food source by marine invertebrates and fish in the mangal (Dighton et al., 2005).

This high protein material additionally serves as the foundation of the microbial loop supporting the sustainability of many economically important fish and crustaceans. Fell and Master (1980) examined the role of marine fungi in the mangrove environment in south Florida. They created a model to describe the effect fungi had upon the release of nitrogen and carbon. They found that fungi increased carbon loss from leaf litter by 3% and that inorganic nitrogen was not removed in the absence of fungi (Fell & Master, 1980). They concluded that leaf litter had low nitrogen levels due to early invertebrate colonization of the leaves (Fell & Master, 1980).

1.7 Geographic Distribution of Marine Fungi

Hughes (1974) defined four geographic zones of marine fungal distribution; the temperate, tropical, sub-tropical, and cold water zones. According to Hughes scheme, most marine fungi are grouped as pantemperate or pantropical, however there is no evidence to suggest that species are restricted to countries or continents (Jones, 1993).

Bebout *et al.* studied the growth rate of five strains of the marine fungi *Corollospora maritima* Werderm and found significant differences in their response to temperature. Strains were isolated from four different graphics: (1) cosmopolitan, (2) cold water (arctic and antarctic), (3) temperate water, and (4) warm water (tropical and subtropical). At 30°C, strains collected from warm waters grew at higher rates than cold-water isolates. At 10°C, strains collected from cold-waters grew at higher rates then warm water isolates. Finally, strains collected from temperate waters had growth rates that were intermediate between those of cold-and-warm-water strains. It was concluded that strains of *C. maritima* are adapted to grow best near the temperatures at which they are found and collected from in nature (Beboute, Schatz, Kohlmeyer, & Hailbach, 1987)

1.8 Effect of Salinity and Temperature on the Growth and Distribution of Marine Fungi

Because of the near-shore marine environment's fluctuating nutrient, temperature, and salinity levels, it was suggested that only 500-600 species of marine fungus are capable of completing their entire lifecycle in the ocean (dela Cruz et al., 2006). Physiological mechanisms help marine fungi to cope with various abiotic factors including salinity levels, fluctuating pH, light and darkness on mycelial growth and spore germination, and varying seasonal temperatures (dela Cruz et al., 2006). Salinity and temperature appear to be the two major components affecting the diversity and geographic range of marine fungus (Dighton et al., 2005).

1.9 Marine Fungal Physiological Adaptations to Salinity

Halophiles keep their internal environment at a lower water potential relative to the surrounding environment to allow an influx of water. This is typically accomplished by having a

high internal concentration of osmotically active substances. Marine plants and algae do this by accumulating high concentrations of sodium and chloride in a metabolically inert vacuole away from the low-volume salt-sensitive cytoplasm (Hajibagheri, Hall, & Flowers, 1984; Hellebust, 1985). Polyols and monovalent ions such as sodium and potassium help to maintain the intracellular osmotic pressure gradients in *Dendryphiella salina* (Wethered, Metcalf, & Jennings, 1985). Clipson *et.al* (1989, 1990) studied *D. salina* to determine which osmotically active substances helped to sustain the internal low water potential and found that the cells increased their cytoplasmic and vacuolar volume and that these osmotic adjustments were facilitated by the synthesis of organic molecules including mannitol, glycerol, and arbitol.

Palmero Llamas *et. al* (2008) studied the internal osmotic potential of *Fusarium solani* as a function of temperature and found that fungal growth for this particular species was optimum at 35°C and the water potential was also greatest at that temperature. As the temperature dropped so did the water potential and in turn the viability of the cultures. (Palmero Llamas et al., 2008).

Byrne and Jones (1975) investigated the salinity requirements for a variety of higher terrestrial and marine fungi under laboratory conditions. They found that as salinity levels increased, the overall time required for perithecia to develop increased. Additionally they found that salinities greater than 40ppt resulted in smaller perithecia. Low salinities produced relatively lower numbers of perithecia, but these were the greatest in size relative to those observed at higher salinities. The maximum number of perithecia occurred in a salinity range of 10-40 ppt, and these were medium sized perithecia. It was concluded that perithecial growth and development are largely inhibited by increased salinity. Man marine species were able to produce perithecia across a wide range of salinities. The lone exception was *Halosphaeria*

appendiculata which was unable to make asci or ascospores in freshwater media (Byrne & Jones, 1974).

Near shore regions differ in their intertidal amplitude and ambient salinity levels which can affect species diversity. Studies by Jones and Jennings (1964) focused on the ability of marine fungi to grow at various salinity levels. Zoosporic fungi including *Althornia, Haliphthoros* and *Thraustochytrium* species have a sodium requirement for growth (Alderman & Jones, 1971). However, *Schizochytrium* species have been isolated from low salinity mangrove habitats while *Halophytophthora* species have demonstrated a wide salinity tolerance in nature and laboratory tests (Nakagiri, Newell, Ito, Tan, & Pek, 1996). This suggests that marine fungi are adapted to a wide range of salinity such as occurs in a mangrove environment. Jennings (1983) later concluded that higher mycelial marine fungi do not appear to have a sodium requirement, but rather a combination of factors allows these fungi to grow in the marine environment. It was found that marine fungi, "…can tolerate concentrations of ions present in seawater and prefer the alkaline pH of seawater" (Jones, 2000).

Several studies investigating the effect of salinity on fungal growth focused primarily on vegetative growth. Harrison and Jones (1975) showed that freshwater saprolegniaceous fungi were unable to reproduce at salinities greater than 30%. In contrast, Padgett (1978) found that the saprolegniaceous fungi were able to survive at salinities less than 18%, but they were, "not physiologically adapted to, nor morphologically active in highly saline environments" (Padgett, 1978).

1.10 Effect of Salinity on the Distribution of Marine Fungi

Typically, the effects of salinity on fungal growth are studied at controlled temperatures. Ritchie (1957) first described the *"Phoma"* pattern which revealed the interrelationship between temperature and salinity. Two species of imperfect fungi (*Phoma herbarum and Pestalotia aletridis*) were isolated from Limon Bay, Panama and another (*Lulworthia medusa* var. *biscaynia*) was taken from San Juan, Puerto Rico. The individual isolates were cultured at temperatures of 6°C,25°C, and 37°C on agar media at controlled salinities of 0.8%, 1.5%, 2.3%, 3.0%, 6.0%, or 9.0%. It was observed that the dual factors of temperature and salinity influenced the overall growth rate. The fastest growth rate occurred at 25°C regardless of the salinity, however optimal growth was seen at combinations of low salinity and low temperature and at high salinity and high temperature (Ritchie, 1957). However, the combination of low temperature and high salinity or high temperature and low salinity resulted in the inhibition of fungal growth. This duality of temperature and salinity affecting the role of fungal growth is now referred to as the "*Phoma"* pattern.

Shearer (1972) studied the distribution of fungi along a salinity gradient in the Patuxent River of Maryland. Shearer found distinct differences in fungal composition at varying parts of the river. Additionally, as salinity levels increased, the ratio of Ascomycetes to Fungi Imperfecti increased. This shift in fungal composition in relation to increased salinity was also observed by Höhnk (1956) in a North Sea estuary and Hughes (1960) which lead to the conclusion that salinity levels affect the distribution of marine fungi in estuarine areas.

Kirk and Schatz (1980) studied the effects of salinity on wood and aquatic vegetation in the Back Bay Wildlife Refuge in Virginia. Submerged wood samples were collected in an embayment area that was isolated from the ocean by barrier dunes. At the beginning of the study a salinity gradient had been established because sea water was being pumped into the bay for

wildlife management (Kirk & Schatz, 1980). In the presence of the artificial salinity gradient 14 Ascomycetes, 10 Dueteromycetes, and 8 non-marine fungus were identified. Once sea water pumping ceased, 5 Ascomycetes, 10 Dueteromycetes, and 21 non-marine fungi were identified. The investigators concluded that the diversity of marine fungi decreased with the concomitant decrease in mean salinity along the gradient (Kirk & Schatz, 1980).

In 2000, Mickle studied the distribution of marine fungi along the salinity gradient of the New River in South Florida. Soft and Hardwood panels were submerged along the salinity gradient at five stations and studied throughout the year. Thirteen species were identified including 9 Deuteromycetes and 4 Ascomycetes. A majority of the species occurred at low salinities. Mickle (2000) found that temperature and pH had little effect on the distribution, however the fungi demonstrated a preference for the softwood.

Jones and Puglisi (2006) reported on the occurrence of marine fungi at Biscayne Bay, Florida. They found the diversity of marine fungi was similar to that seen in other tropical areas. They did not study the seasonal occurrence of fungi in the area. Studying the distribution of marine fungi in relation to seasonal temperature and salinity changes allow for a better understanding of their physiological requirements, dispersal, and life-cycles. These observations can also be of importance if new host substrates are introduced into the area (Volkmann-Kohlmeyer & Kohlmeyer, 1993).

1.11 Project Overview and Objectives

Due to the paucity of information regarding the distribution of higher marine fungi along the west (Gulf) coast of Florida, the aim of this study was to investigate the distribution of marine fungi in relation to salinity and seasonal temperature variation at Rookery Bay National

Estuarine Research Reserve in Naples, Florida. Species identification, substrate preference, and physical parameters including temperature and salinity were also investigated. Data was analyzed in a statistical way to examine whether salinity and variation in seasonal temperature significantly contributed to fungal colonization of wood substrates and their ability to reproduce on those substrates.

2. Materials and Methods

The seasonal occurrence and activity of higher filamentous marine fungi were examined at the Rookery Bay National Estuarine Research Reserve located in, Naples, Florida. Sampling of three study sites started in April 2009 and continued through April 2010. The three study sites were chosen to reflect low salinity levels as seen in an area of fresh water runoff- Station One; Intermediate or estuarine salinity levels –Station Two; high or oceanic salinity levels–Station Three (Table I). Station one was established at an upper area of Henderson Creek which has an altered flow pattern due to a freshwater source. Station two was established approximately 3km downstream from the Manatee Basin Tributary where intermediate salinity levels occur (around 15 ppt) around Henderson Creek (Rousu, 2000). Station Three was established at the lower area of Henderson Creek where salinity levels of 36.7 ppt are regularly recorded (Figure I).

Test blocks of *Pinus* sp. and *Quercus* sp. (10x10x5cm) were threaded in alternating sets on weather resistant rope. Paired panels were separated by two zip ties on the rope. Lines were secured with zip ties to floating docks at each station and submerged below the water line to prevent blocks from being exposed to the atmosphere. One pair of blocks was retrieved from each site after submergence periods of 3, 6, 9, and 12 months.

The salinity and temperature of Henderson Creek was analyzed using real time data provided by the National Estuarine Research Reserve System (NERR). Water quality data was compiled from data obtained in previous years to detect any major fluctuations or disturbances in the water quality.

Removed blocks were cleaned of fouling organisms and incubated at room temperature (23°C) in sealed, moist plastic containers approximately 7.5cm in depth and examined periodically for fungi. Mycelia and reproductive structures were removed from superficial layers using a scalpel, placed in a drop of distilled water on a microscope slide, depressed with a cover slip and observed under a dissecting microscope at 40x magnification. Fungi were identified using mainly the keys by Kohlmeyer, J., & Kohlmeyer, E. (1979), Jones (2009), and Kohlmeyer. J, & Volkmann-Kohlmeyer, B. (1991). If reproductive structures were not found immediately after collection, materials were incubated at room temperature and examined periodically.

For each fungal species the per cent frequency of occurrence was determined (Table II). The diversity of marine fungi in each location was assessed based on the diversity indices and evenness.

3. Results

Over a one year period (April 2009 to April 2010), a total of one-hundred-and-sixteen higher filamentous marine fungi were identified along the salinity gradient at Rookery Bay (Table 3). The Ascomycetes were the dominant group, represented by seventy-two species (61.5%). Additionally forty-one Deuteromycetes (35.0%) and four Basidiomycete species were identified (0.03%). While dominant species were observed in each 4 month test period, the majority of species were found only once or twice during the study. Dominant species, defined as having a total frequency of occurrence above 80% displayed the ability to successfully

sporulate over a wide range of salinities and temperatures. The dominant species seen in this study were *Biflua physasca* (Jørg. Koch & E.B.G Jones), *Halorosellinia oceanica* (S. Schatz) Whalley, E.B.G. Jones, K.D. Hyde & Lassøe), *Periconia prolifica* (Anastasiou), and *Trichocladium alopallonellum* ((Meyers & R.T. Moore) Dixon).

The total species number, percent species, and species diversity steadily decreased during the course of the study (Table 4). A maximum of seventy-three species was identified in the first test period (4/1/2009-1/7/2009) and a minimum of 64 in the fourth test period (4/1/2009-4/7/2010) (Table 4). Over the course of the study 46 species were observed at all three sites over a salinity range of 5-36ppt. Theses species did not necessarily occur at a high frequency but displayed the ability to sporulate in a wide range of salinities. Thirty-seven of the 116 species were collected at only one site and most of these species were considered to be rare.

Variation in the frequency of occurrence of species was most noticeable between study stations as opposed to test periods. Certain species exhibited relatively higher or lower frequencies at the high salinity site suggesting that salinity contributed to that testing site's species composition and diversity. The highest total frequency of Ascomycetes and Basidomycetes occurred at Bear Hammock (moderate salinity) while the highest frequency of Deuteromycetes was seen at the Visitor Center (low salinity) (Figure 5). The occurrence Ascomycetes generally increased over the course of the study, whereas the occurrence of both Basidiomycetes and Deuteromycetes generally decreased (Figure 4). The ratio of Ascomycetes and Imperfect fungi also increased from the Visitor Center (low salinity) to the Field Station (high salinity) (Figure 7).

Species richness fluctuated at each site but in general appeared to decrease over the 1-year study period. The Field Station (high salinity) consistently displayed the highest species

richness, while Bear Hammock (moderate salinity) showed the lowest species richness (Table 6). There was an unexpected spike in species richness at the Field Station during the fourth testing period. This was attributed to the appearance of wood borers which created additional substrate area for fungi to colonize and allowed those species access to nutrients deeper in the wood test panels.

While most fungi occurred on both types of test panels (Pine and Oak) twenty-seven species only occurred on a single substrate such as *Alternaria* sp. Seventeen species only colonized the Oak panels (hardwood) while 10 only colonized Pine panels (softwood) A two-sample T-test was used to examine for substrate preference of the fungi collected and produced an insignificant p-value of 0.1534 (Table 7). This suggested that, overall, the fungi collected in my study did not exhibit a particular substrate preference.

There was little variation of water temperature between sites during the study. Thus, water temperature does not appear to have been a contributory factor to species diversity between study sites. Variation in temperature between test periods however did result in overall changes in species composition at each site.

Ambient water temperatures ranged from 11.0^{o}C to 32.5^{o}C over the one year test period (Table 8). The average seasonal temperature was 22°C. Effects of temperature were most noticeable during the third and fourth sampling periods (4/1/2009-1/7/2010; 4/1/2009-4/7/2010) when the temperature at each station was lowest. The total species diversity and richness also reached a minimum at this time. The sudden decrease in temperature was expected during that period and has been reported in previous studies from south Florida as well (Adams, 2003b; Vogel et al., 2008). During these periods identification of fungi in the lab also took longer relative to the other test periods. In the first initial weeks of incubation only a few species were

identified including *Periconia prolifica* and *Halorosellinia oceanica*. Following a longer incubation period more fungi began to fruit on the wood which may have been due to the increased temperature in the lab relative to the *in situ* temperature. The increased temperature may have allowed for more a optimal sporulation environment.

While each species of fungus has an optimal growing temperature, most tropical species grow best at around 25°C. The optimum temperature for growth of marine fungi in this study was 30°C and produced the highest diversity and species richness (Table 4). When the temperature reached a maximum of 35°C during the second test period (4/1/2009-10/15/2009) species richness decreased from the first test period. The increase in temperature may have been inhibitory to sporulation for certain species causing lower species diversity.

Salinity was measured at each station on a monthly basis. Salinity appeared to have the greatest effect on species diversity between sites as well as altering the frequency of occurrence of individual species over the 1-year testing period. A salinity gradient was seen with a mean of 11.25-19.75-31.00ppt moving from the Visitor Center, to Bear Hammock, to the Field Station. The Visitor Center is a low salinity environment ranging from 5-20ppt. The site located at Bear Hammock is of moderate salinity ranging from 4-32ppt. The Field Station demonstrated a consistently high salinity marine environment with salinity ranging from 26-36ppt. The minimum salinity was 5ppt and the maximum was 36ppt. All three sites experienced a drop in salinity during the second test period (4/1/2009-10/15/2009) which may be attributed to the annual rainy season that occurred during this period (Table 8, Figure 2).

Previous collections from the lower East coast of Florida were compared to the Naples collection (Table 10). Several of the species reported from the East coast studies were also collected in my study. Certain dominant species identified from the Florida East coast studies

however were not identified in my study. Common dominant species included *Lulworthia* sp. (G.K. Sutherland), *Payosphaeria minuta* W.F. Leong, *Cirrenalia macrocephala* (Kohlm.) Meyers & R.T. Moore), *Trichocladium alopallonellum* (Meyers & R.T. Moore) Kohlm & Volkm.-Kohlm), and *Trichocladium lignincola* Schmidt. The West coast collection had significantly higher number of species as well as a higher species diversity. The south Florida east coast regions exhibited higher ambient water temperatures as well as being affected by hurricane activity during the study period which may have accounted for differences in species composition.

Figure 1. Area of Study Henderson Creek, Naples, Fl.

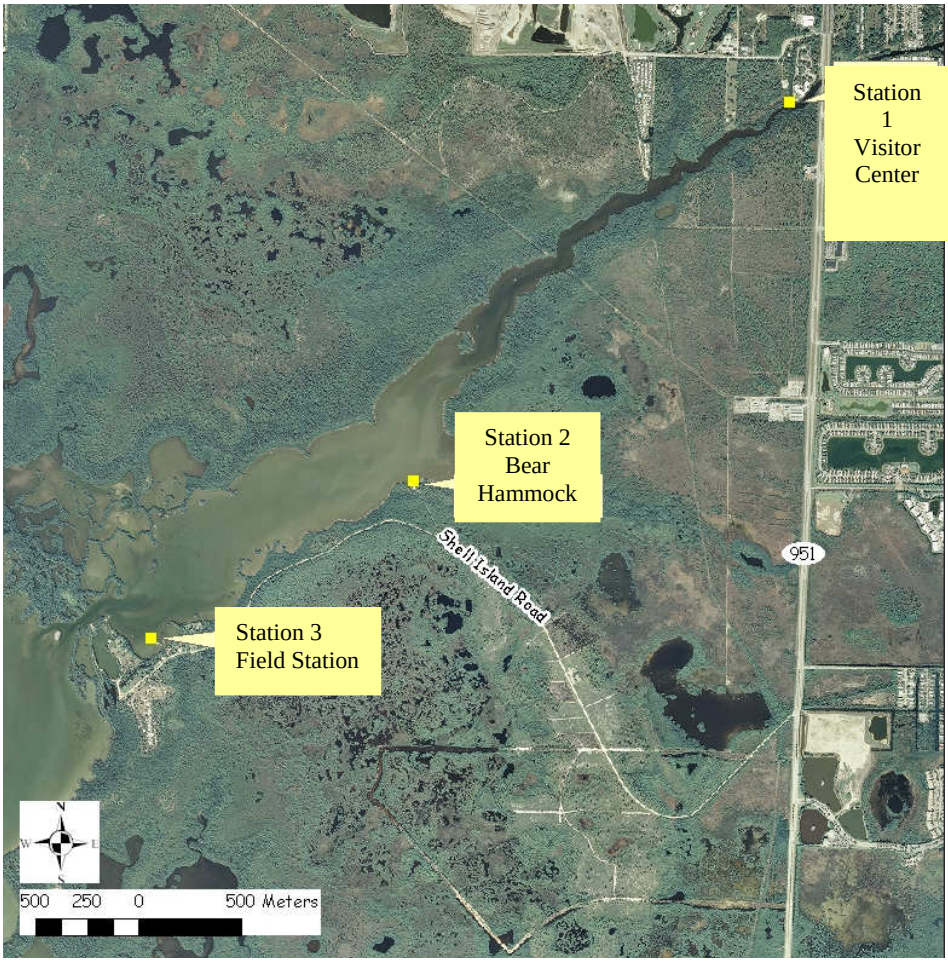

Table 1. Position of Panels

	Latitude N	Longitude W
Station 1 – Visitor Center	26° 02' 57.95" N	81°42' 05.38" W
Station 2- Bear Hammock	26° 01' 56.72" N	81°43' 12.00" W
Station 3- Field Station	26° 01' 32.81" N	81°43' 56.72" W

Table 2. Periods of Panel Submergence

Period 1	Period 2	Period 3	Period 4
4/1/2009-7/9/2009	4/1/2009-10/15/2009	4/1/2009-1/7/2010	4/1/2009-4/7/2010

3.Results

Table 3. Frequency of occurrence and dominant species at each site

Fungi	Total frequency occurrence[a]	Frequency of occurrence (%) Location[b]		
		VC	BH	FS
Ascomycota				
Acrocordiopsis patilii	0.042	--	--	0.125
Aigialus striatispora	0.042	--	--	0.125
Amylocarpus encephaloides	0.375	0.125	0.25	0.75
Aniptodera intermedia	0.042	--	--	0.125
Aniptodera nypae	0.083	--	0.250	--
Argentinomyces naviculisporus	0.042	--	--	0.125
Arthrobotrys arthrobotryoides	0.042	0.125	--	--
Astrocystis nypae	0.417	0.25	0.5	0.5
Belizeana tuberculata	0.167	--	0.25	0.25
Bicrouania maritima	0.75	1.000	0.5	0.75
*Biflua physasca**	1.000	1.000	1.000	1.000
Byssothecium obiones	0.042	--	0.125	--
Capillataspora corticola	0.083	--	--	0.25
Carbosphaerella leptosphaerioides	0.042	--	--	0.125
Carbosphaerella pleosporoides	0.042	--	--	0.125
Caryospora australiensis	0.042	--	--	0.125

Caryosporella rhizophorae	0.042	--	0.125	--
Ceriosporopsis halima	0.167	0.125	0.125	0.25
Corollospora californica	0.167	0.125	0.125	0.25
Dactylospora haliotrepha	0.083	0.125	--	--
Dactylospora mangrovei	0.125	0.125	--	0.25
Decaisnella formosa	0.083	0.125	--	--
Didymella avicenniae	0.083	0.125	0.125	--
Eiona tunicata	0.333	0.375	0.25	0.375
Gymnascella littoralis	0.583	0.625	0.5	0.625
Halonectria milfordensis	0.042	--	--	0.125
*Halorosellinia oceanica**	1.000	1.000	1.000	1.000
Halosphaeria trullifera	0.042	--	0.125	--
Halosarpheia unicellularis	0.625	0.5	0.75	0.625
Helicascus kanaloanus	0.042	--	0.125	--
Hypophloeda rhizospora	0.042	0.125	--	--
Kallichroma glabrum	0.167	0.125	0.25	0.125
Kirschsteiniothelia maritima	0.125	0.25	0.125	--
Kohlmeyeriella tubulata	0.042	--	--	0.125
Lautospora gigantea	0.042	0.125	--	--
Leptosphaeria australensis	0.875	1.000	0.75	0.875
Leptosphaeria pelagica	0.042	--	0.125	--
Lignincola laevis	0.25	0.25	0.25	0.25
Lindra hawaiiensis	0.5	0.375	0.5	0.625
Lindra inflata	0.583	0.25	0.875	0.625
Lulwoana uniseptata	0.042	0.125	--	--
Lulworthia floridana	0.208	0.125	0.125	0.375
Lulworthia lindroidea	0.042	0.125	--	--
Manglicola guatemalensis	0.083	0.125	0.125	--
Marinosphaera mangrovei	0.042	--	--	0.125
Massarina lacertensis	0.083	0.25	--	--
Massariosphaeria typhicola	0.083	0.125	0.125	--
Moana turbinulata	0.583	0.5	0.5	0.75
Morakotiella salina	0.042	--	--	0.125
Nais inornata	0.042	--	0.125	--
Nemania maritima	0.042	0.125	--	--
Neptunella longirostris	0.083	0.125	0.25	--
Nereiospora comata	0.042	0.125	--	--
Okeanomyces cucullatus	0.25	--	0.375	0.375
Paraliomyces lentifer	0.708	0.875	0.75	0.5
Patellaria atrata	0.083	--	--	0.25
Payosphaeria minuta	0.792	0.75	0.75	0.875

Pedumispora rhizophorae	0.083	--	0.25	--
*Phaeosphaeria capensis**	0.917	1.000	0.875	0.875
Phaeosphaeria halima	0.042	0.125	--	--
Phaeosphaeria orae-maris	0.083	--	0.25	--
Phomatospora nypicola	0.042	--	--	0.125
Pseudolignincola siamensis	0.083	--	0.125	0.125
Pyrenographa xylographoides	0.5	0.625	0.375	0.5
Quintaria lignatilis	0.042	--	--	0.125
Rhizophila marina	0.042	--	--	0.125
Sphaerulina orae-maris	0.042	0.125	--	--
*Thalassogena sphaerica**	0.958	0.875	1.000	1.000
Trematosphaeria mangrovei	0.083	0.25	--	--
Ulocladium atrum	0.083	0.250		
Verruculina enalia	0.25	0.375	0.125	0.250
Zopfiella marina	0.083	--	0.25	--
Basidiomycota				
Digitatispora marina	0.042	--	--	0.125
Haloaleurodiscus mangrovei	0.167	--	0.125	0.375
Rostrupiella danica	0.042	--	--	0.125
Deuteromycetes				
Asteromyces cruciatus	0.042	0.125	--	--
Acremonium tubakii	0.417	0.25	0.375	0.625
Allescheriella bathygena	0.042	0.125	--	--
Alternaria sp.	0.125	0.125	0.125	0.125
Amarenomyces ammophilae	0.042	--	--	0.125
Amorosia littoralis	0.542	0.375	0.500	0.750
Botryophialophora marina	0.042	0.125	--	--
Cirrenalia basiminuta	0.833	0.750	0.875	0.875
Cirrenalia fusca	0.667	0.750	0.750	0.500
Cirrenalia macrocephala	0.500	0.625	0.75	0.125
Cirrenalia pseudomacrocephala	0.250	0.125	0.25	0.375
Cirrenalia pygmea	0.208	0.125	0.25	0.25
Cirrenalia tropicalis	0.625	0.625	0.875	0.375
Cladosporium algarum	0.125	0.375	--	0.25
Cumulospora marina	0.583	0.875	0.500	0.375
Cumulospora varia	0.417	0.875	0.250	0.125
Dictyosporium pelagicum	0.083	0.125	--	0.125
Dinemasporium marinum	0.125	0.125	0.250	--
Diplodia orae-maris	0.5	0.750	0.250	0.5

Emericellopsis maritima conidium	0.125	--	--	0.375
Endophragmia dimorphospora	0.208	--	0.25	0.25
Halenospora varia	0.25	0.25	0.125	375
Halocyphina villosa	0.333	0.625	0.375	--
Heliscella stellatacula	0.083	--	--	0.25
Macrophoma sp.	0.167	0.125	0.25	0.125
Metarhizium album	0.625	0.625	0.75	0.5
Monodictys pelagica	0.125	--	--	0.25
*Periconia prolifica**	1	1	1	1
Phialophorophoma litoralis	0.083	0.25	--	--
Phoma	0.625	0.625	0.625	0.625
Remispora galerita	0.042	0.125	--	--
Rhabdospora avicenniae	0.042	0.125	--	--
Stemphylium maritimum	0.083	0.125	0.125	--
Trichocladium achrasporium	0.667	0.625	0.625	0.75
*Trichocladium alopallonellum**	1	1	1	1
Trichocladium constrictum	0.833	0.875	0.875	0.75
Trichocladium lignicola	0.708	0.75	1	0.375
Trichocladium melhae	0.333	0.5	0.375	0.125
Varicosporina ramulosa	0.042	0.125	--	--
Zalerion maritima	0.25	0.375	0.25	0.125
Zopfiella latipes	0.125	--	0.125	0.125

[a] See Materials and Methods for details

[b]Locations: Visitor Center (low salinity); Bear Hammock (moderate salinity); Field Station (high salinity). Highlighted species denotes those occurring at all three stations

* Denotes dominate species

Table 4. Species Richness, diversity, and evenness of marine fungi recorded from 3 locations over one year along Henderson Creek, Naples FL

Location	Species Richness	Percent Species [a]	Diversity Index		Shannon Evenness
			Simpson	Shannon	
Visitor Center	78	67.2	0.0179500	3.9790	0.9133
Bear Hammock	75	64.7	0.0178140	3.9815	0.9222
Field Station	77	66.4	0.0178900	3.9785	0.9159

[a] Considering 116 as 100%

Table 5. Total Species Richness, diversity, and evenness of marine fungi recorded from 3 locations along Henderson Creek, Naples FL

Incubation Period (months)	Species Richness	Percent Species [a]	Diversity Index		Shannon Evenness
			Simpson	Shannon	
3	73	62.9	0.01430	4.0857	0.9523
6	70	60.3	0.01580	4.0150	0.9450
9	66	56.9	0.15670	3.9974	0.9541
12	64	55.2	0.01657	3.9529	0.9505

[a] Considering 116 as 100%

Table 6. Seasonal Species Richness, diversity, and evenness of marine fungi recorded from 3 locations along Henderson Creek, Naples FL

Location	Species Richness	Percent Species [a]	Diversity Index		Shannon Evenness
			Simpson	Shannon	
3 Months					
Visitor Center	44	58.7	0.00952	3.7247	0.98427
Bear Hammock	41	54.7	0.01903	3.6564	0.98459
Field Station	48	64.0	0.00859	3.8116	0.98459
6 Months					
Visitor Center	**41**	**57.7**	0.01028	**3.6541**	0.98399
Bear Hammock	**41**	**57.7**	0.01101	**3.6554**	0.98432
Field Station	**45**	**63.4**	0.00818	**3.7486**	0.98474
9 Months					
Visitor Center	42	63.6	0.02720	3.5343	0.94559
Bear Hammock	40	60.1	0.01065	3.6296	0.98391
Field Station	44	66.7	0.00904	3.7247	0.98420
12 Months					
Visitor Center	40	58.8	0.01129	3.6322	0.98465
Bear Hammock	33	48.5	0.01168	3.6076	0.98473
Field Station	54	79.4	0.00704	3.9118	0.98066

[a]considering 75 as 100% at 3 months; 71 as 100% at 6 months; 66 as 100% at 9 months; 68 as 100% at 12 months.

Table 7. t-Test: Two-Sample Assuming Equal Variances to determine significance of substrate preference

	Pine	*Oak*
Mean	46	43.75
Variance	4.67	11.58
Observations	4	4
Pooled Variance	8.125	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.1163	
P(T<=t) one-tail	0.1534	
t Critical one-tail	1.9432	

Table 8. Salinity and Temperature Statistics during study

Salinity (ppt)				
Location	**Mean**	**Maximum value**	**Minimum Value**	**Standard Deviation**
Visitor Center	11.25	20	5	7.53
Bear Hammock	19.75	32	4	14.04
Field Station	31.00	36	26	5.00
Temperature (C)				
Visitor Center	25.823	33	11	11.22
Bear Hammock	26.50	35	13	11.09
Field Station	24.88	32.5	11	10.90

Figure 2. Seasonal Salinity Change along Henderson Creek

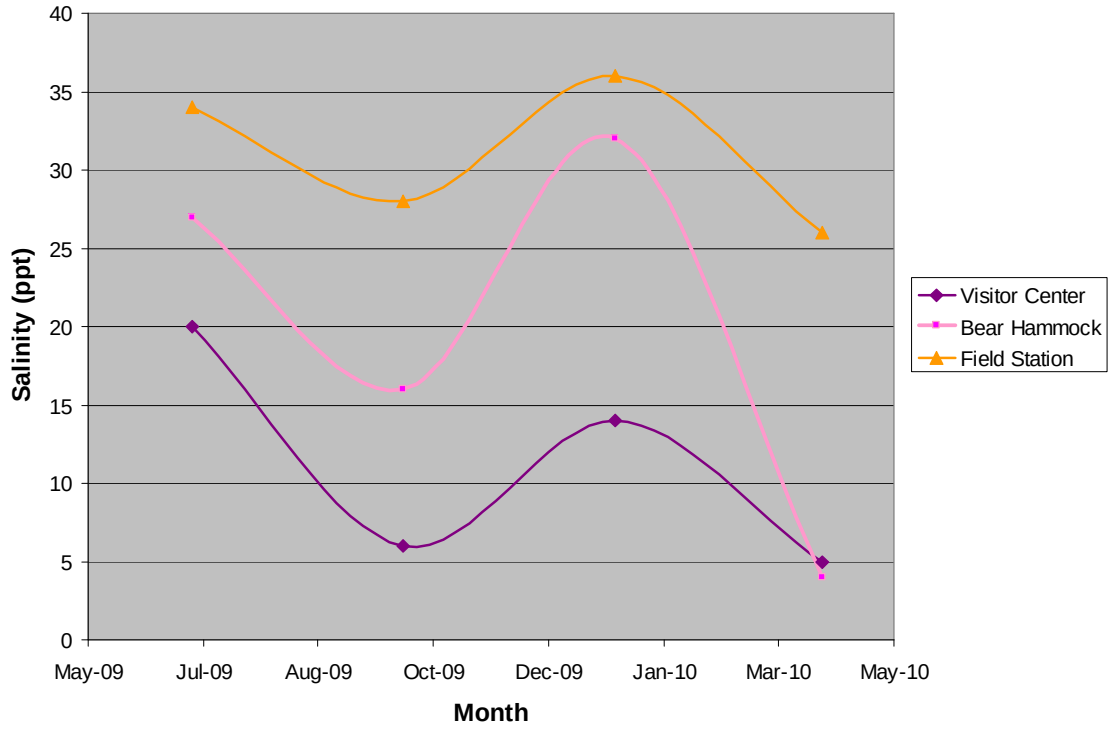

Figure 3. Seasonal Ambient Water Temperature along Henderson Creek

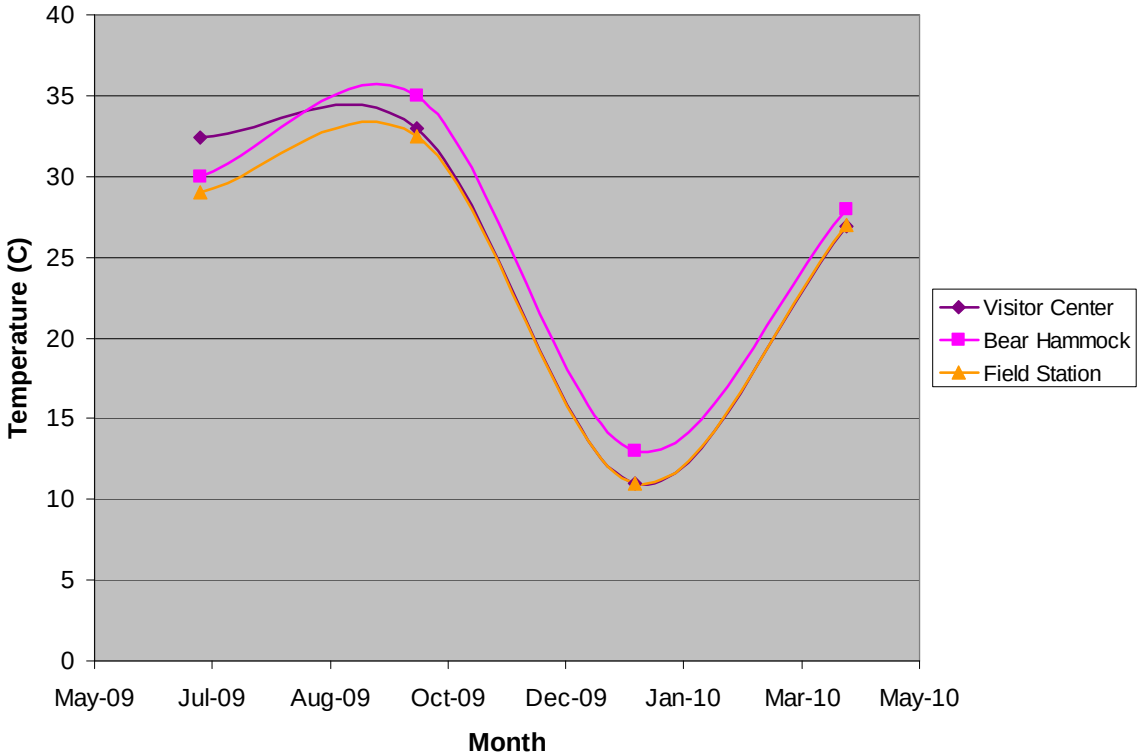

Figure 4. Ratio of Ascomyectes to Imperfect Fungi at each Site and Average Salinity

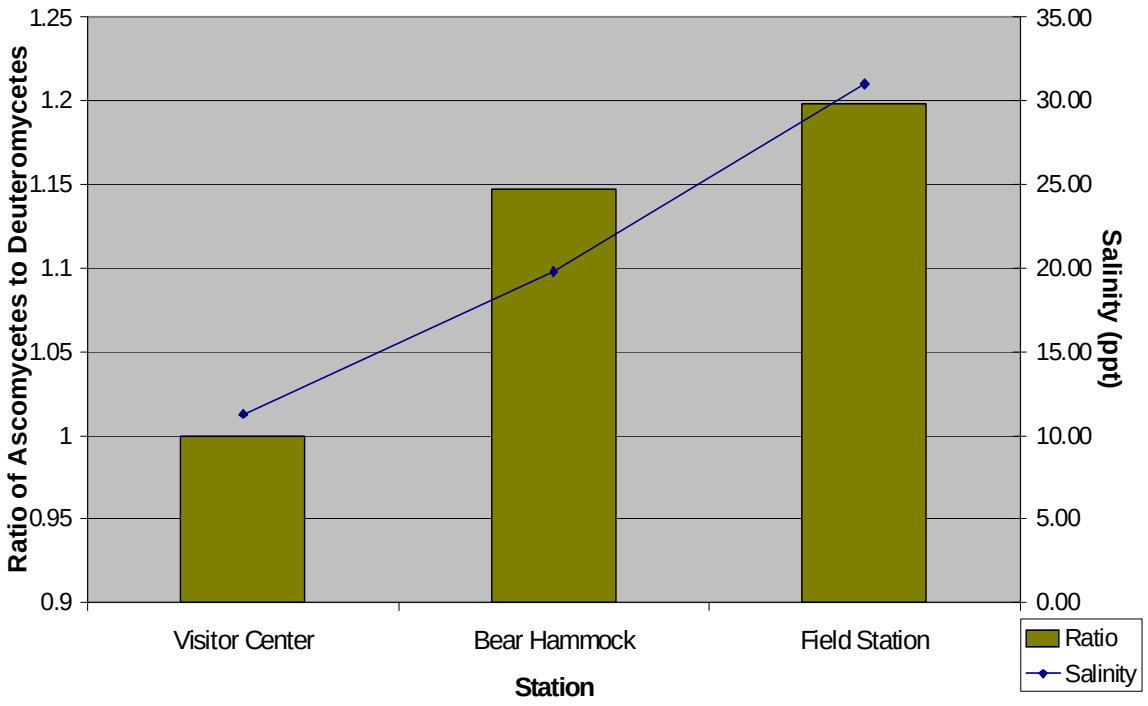

Figure 5. Seasonal number of species of Ascomycetes, Basidiomycetes, and Deuteromycetes

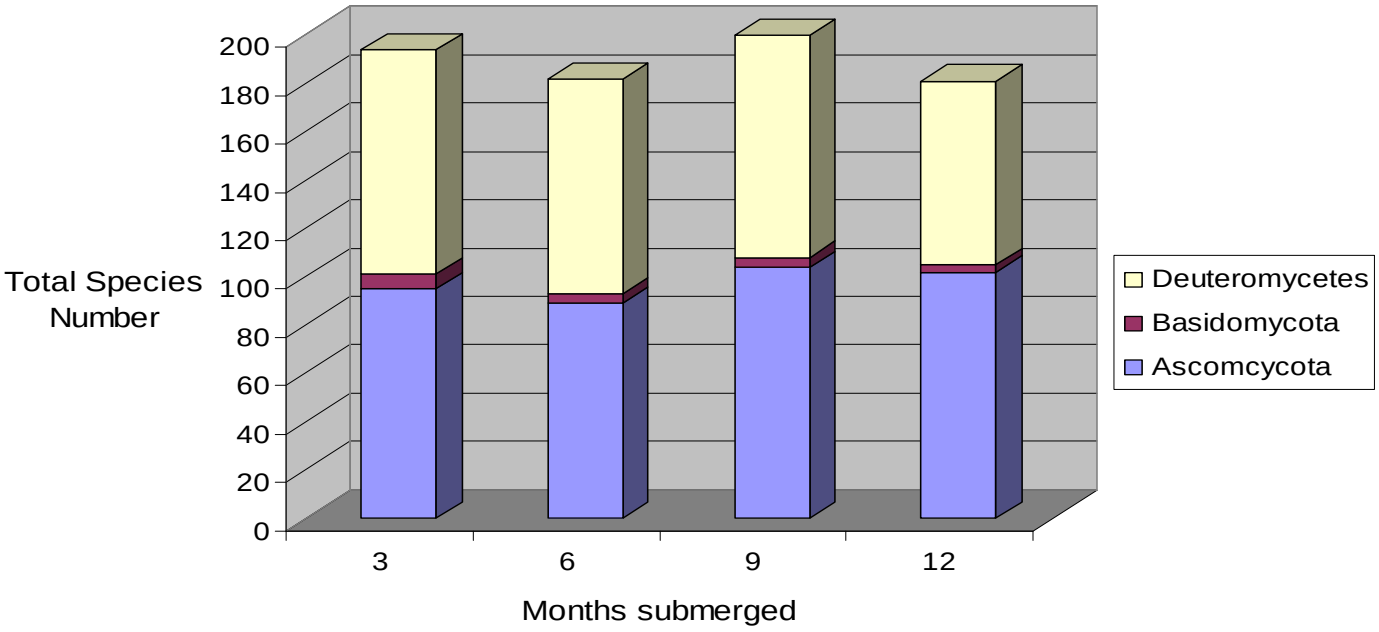

Figure 6. Total Number of Species of Ascomycetes, Basidiomycetes, and Deuteromycetes at each Site

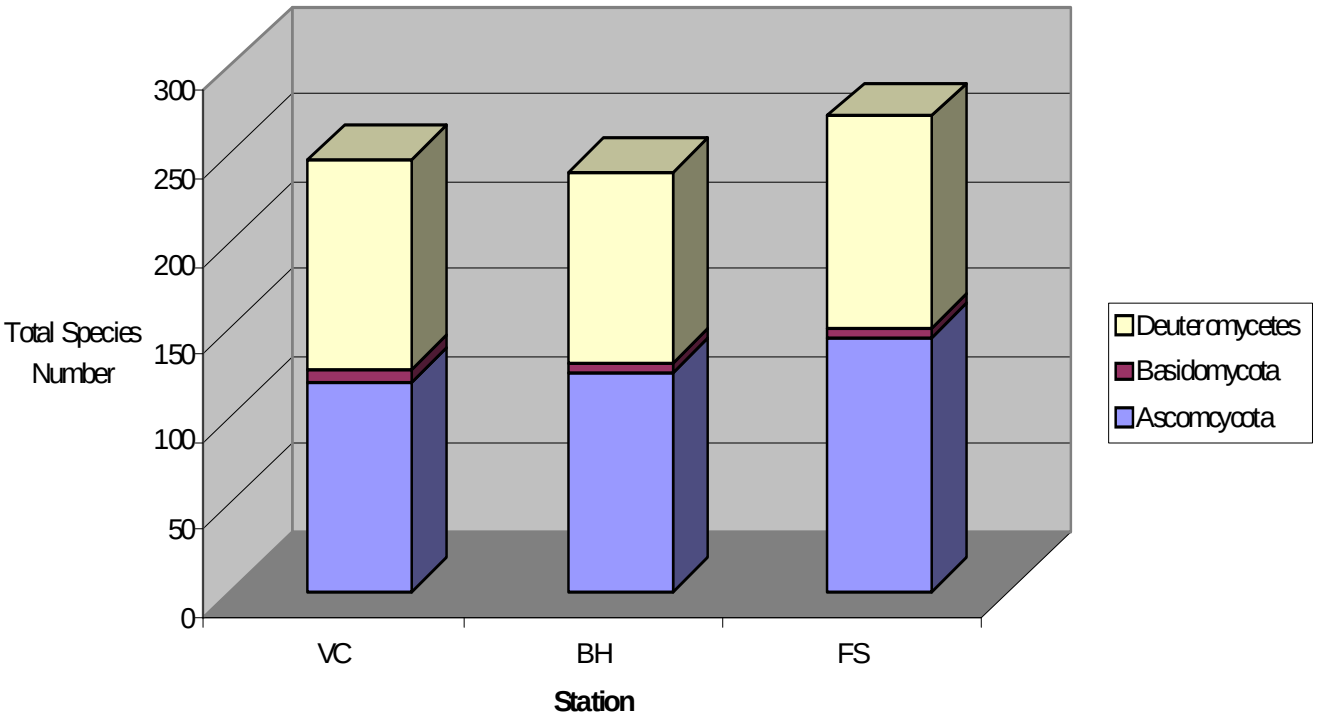

Figure 7. Seasonal Total Number of Species at Low Salinity Site (Visitor Center)

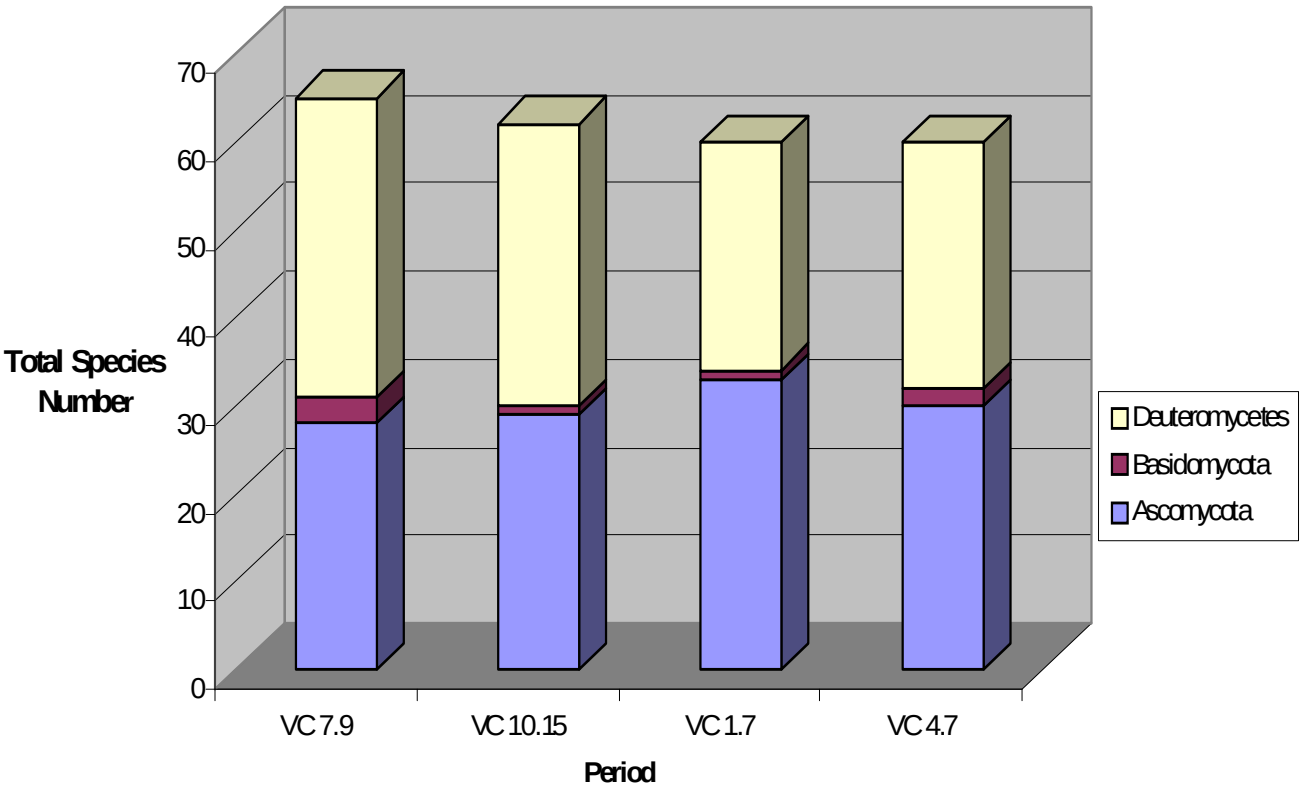

Figure 8. Seasonal Total Number of Species at Moderate Salinity Site (Bear Hammock)

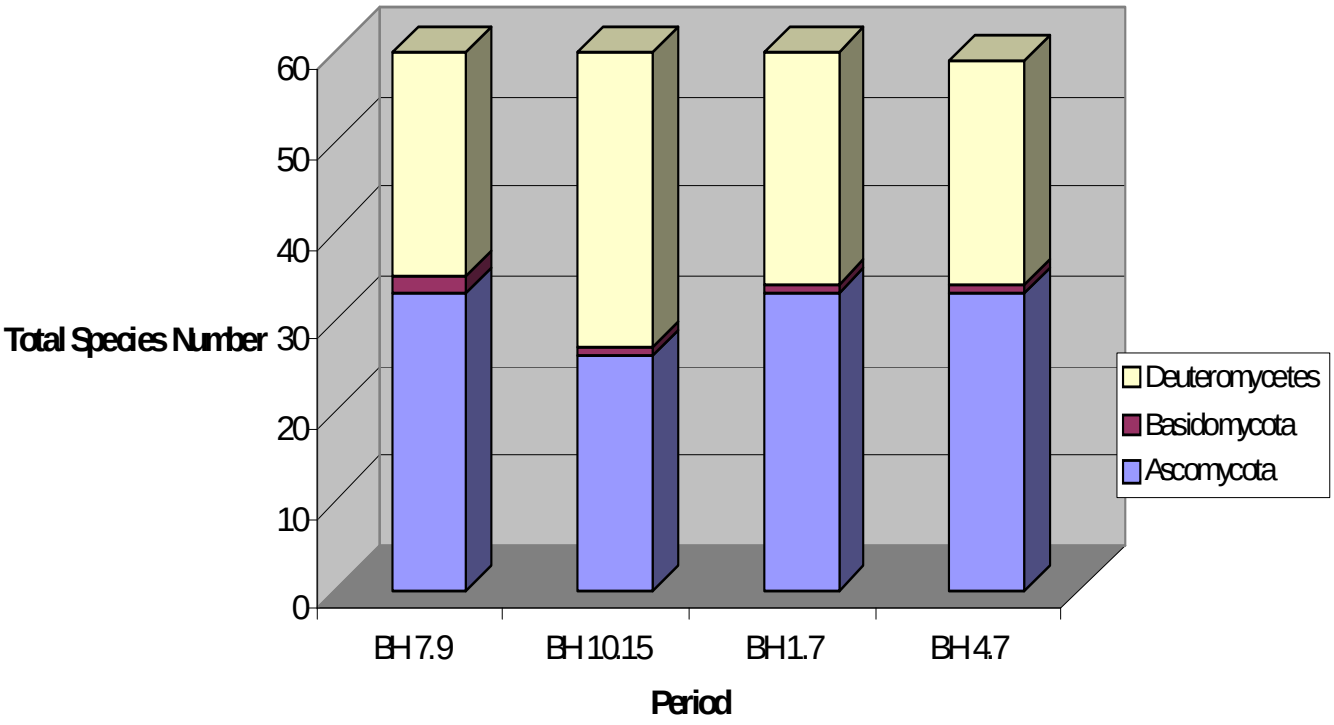

Figure 9. Seasonal Total Number of Species at High Salinity Site (Field Station)

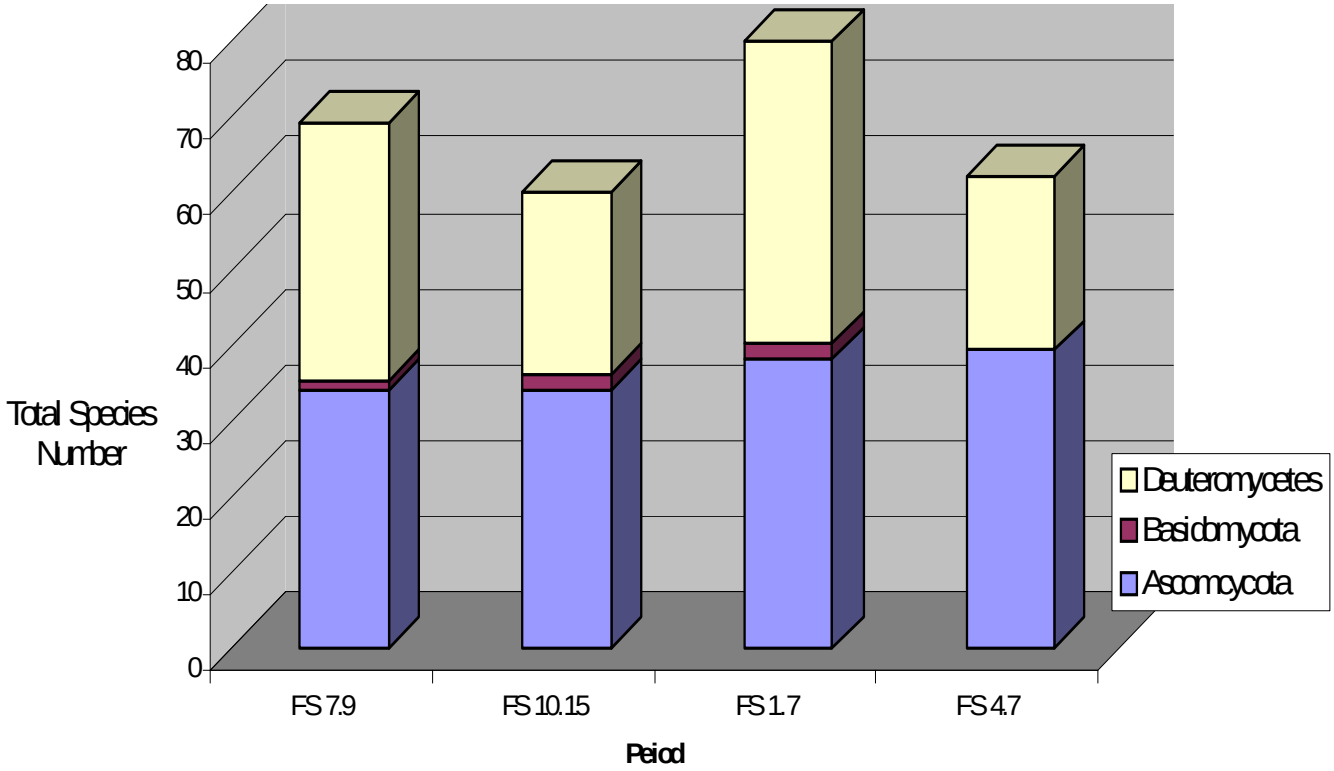

Table 9. Comparison of Species to Florida east coast collections

2000, New River; Broward County, FL (Mickle) [a]N= 13		**2002,Whiskey Creek; Dania Beach, FL** (Vogel) [a]N =34		**2005, Loxahatchee River; Jupiter, FL** (Adams) [a]N =9		**2005, Loxahatchee River; Jupiter, FL** (Kukich) [a]N =23	
Ascomycetes		**Ascomycetes**		**Ascomycetes**		**Ascomycetes**	
Lulworthia sp.		*Aigialus parvus*		*Caryosporella rhizophorae*	X	*Didymosphaeria lignomaris*	
Nais sp.		*Ascocratera manglicola*		*Hypoxylon oceanicum*	X	*Halosphaeria quadricomuta*	
Halosphaeria quadricornuta		*Ascomycete I*		*Lignincola laevis*	X	*Halosarphea unicaudata*	
Verrculina enalia	X	*Ascomycete II*		*Lulworthia spp.*	X	*Leptospheria omameris*	
		Capnodium sp.		*Marinosphaera mangrovei*	X	*Leptosphaeria peruviana*	
		Carbosphaerella pleosp.	X	*Pestalotia* sp.		*Lignincola laevis*	X
		Ceriosporopsis sp.	X	*Quintaria lignatilis*	X	*Lulworthia* sp.	X
		Corollospora maritima	X	*Thalassogena sphaerica*	X	*Nais inornata*	X
		Cucullosporella mangrovei				*Payosphaeria minuta*	X
		Halosarpheia fibrosa				*Savoryella paucisora*	
		Halosphaeria quadricornuta				*Thalassogena sphaerica*	X
		Halorosellinia oceanica	X			*Verrculina enalia*	X
		Leptosphaeria australiensis	X			*Zopfiella marina*	X
		Lineolata rhizophorae					
		Lulworthia grandispora					
		Massarina velatospora					
		Mycosphaerella pneumato.					
		Saaaromyces glitra					
		Phaeosphaeria gessneri					
		Rhizophila marina					
		Savoryella lignicola					
		Verruculina enalia	X				
		Zopfiella marina	X				

2000, New River; Broward County, FL (Mickle) [a]N= 13		**2002,Whiskey Creek ; Dania Beach, Fl** (Vogel) [a]N =34		**2005, Loxahatchee River; Jupiter, FL** (Adams) [a]N =9		**2005, Loxahatchee River; Jupiter, FL** (Kukich) [a]N =23	
Basidiomycetes		**Basidiomycetes**		**Basidiomycetes**		**Basidiomycetes**	
N/A		*Halocyphina villosa*	X	N/A		*Halocyphina villosa*	X
Deuteromycetes		**Deuteromycetes**		**Deuteromycetes**		**Deuteromycetes**	
Cirrenalia macrocephala	X	*Cirrenalia sp.*	X	*Phoma* sp.	X	*Cirrenalia macrocephala*	X
Alternaria sp.	X	*Cladosporium sp.*	X			*Cirrenalia tropicalis*	X
Aspergillus sp.		*Cytospora rhizophorae*				*Conidia (terrestrial)*	
Fusarium sp.		*Epicoccum sp.*	X			*Humicola alopallonella*	X
Humicola alopallonella	X	*Trichocladium alopallonellum*	X			*Perioconia prolifica*	X
Penicillium sp.		*Monodictys pelagica*	X			*Stemphylium gracilariae*	
Periconia prolifica	X	*Periconia prolifica*	X			*Trichocladium lignincola*	X
Trichocladium achrasporum	X	*Trichocladium achrasporum*	X			*Zalerion maritima*	X
		Zalerion maritimum	X			*Zalerion varium*	X

X signifies the fungus was present in the West coast collection

[a]Denotes the total number of species recorded

Highlighted species denote dominate species (dominate species not recorded by Vogel (2002))

4. Discussion

4.1 Comparison of Species Composition to past surveys

Overall, the species composition of the fungi identified in my study is similar not only to previous studies conducted on the Florida east coast but also to studies reported from other tropical and subtropical mangrove forests. The frequency of occurrence of marine fungi in mangrove areas has been reported in earlier tropical-subtropical studies from India (Prasannarai & Sridhar, 2003), Taiwan (Chen & Tzean, 2010), Malaysia (Jones & Alias, 2000), Belize (Volkmann-Kohlmeyer & Kohlmeyer, 1993) West Atlantic (J. Kohlmeyer, 1980b), Florida (Adams, 2003b; Vogel et al., 2008) and several other areas in the Indian Ocean as well as Southeast Asia. Frequently occurring fungi reported from different mangrove areas across the globe include *Antennospora quadricornuta, Dactylospora haliotrepha, Eutypa bathurstensis, Halocryphone villosa, Halorosellinia oceanica, Halosarpheia marina, Kallichroma tethys, Leptosphaeria australiensis, Lophiostoma mangrovei, Lulwortiha grandispora, Lulworthia* sp., *Rhizophila marina, Savoryells longispora,* and *Verrculina enalia* (Sarma & Hyde, 2001).

In this study, *Trichocladium alopallonellum, Periconia prolifica, Halorosellinia oceanica, Biflua physasca, Thalassogena sphaerica,* and *Phaeosphaeria capensis* were collected at each study site. These species have been reported as being frequently found in other mangrove habitats in Tai Ho Bay (Jones et al., 2006), Hawaii (J. Kohlmeyer, 1969), and Brunei (Hyde,1988).The consistent identification of these species in mangrove areas on a global basis indicates that there is a core group of marine fungi that are common colonizers in tropical and subtropical mangrove forests. Species of *Lulworthia* and *Leptosphaeria* have additionally been reported as common colonizers in mangrove areas (Borse, 1988; K. D. Hyde, 1986; Jones, Uyenco, & Follosco, 1988). In this study *Leptosphaeria australensis* was a common colonizer at all three stations (occurring 87.5%) however species of *Lulworthia* only had a total frequency of

occurrence of 20%. *Lulworthia* spp. occurred most often at the high salinity site suggesting that species of this genus may have a high salinity requirement for growth and reproduction.

Investigations of higher marine fungi in The New River, Broward County (Fraser, 2000), Whisky Creek, Dania Beach (Vogel, 2002) and Loxahatchee River, Jupiter (Kukich, 2005) reported *Halosphaeria quadricornuta* (Crib and Crib), *Verruculina enalia* ((Kohlm.) Kohlm. & Volkm.-Kohlm), *Nais glitra* (Crane and Shearer), *Savoryella paucispora* (Cribb and Cribb), *Cirrenalia macrocephala (*(Kohlm.) Meyers & R.T. Moore), *Payosphaeria minuta* (W.F. Leong), and *Trichocladium lignincola* (I. Schmidt) to be dominant species. In this study, *H. quadricornuta* (4.2%), *V. enalia* (25%), *N. glittra* (4.2%) occurred at comparatively lower frequencies. *Savoryella paucispora* was not observed in my study. Differences in species composition relative to other mangrove habitats may be attributed to varying environment parameters including oxygen availability, pH, nitrogen, salinity, temperature, and the condition or age or the wood that was collected. In the study by Mickle (2002) for example, the minimum annual temperature was 20.1°C compared to a minimum of 11°C in my study. Likewise, Kukich (2005) recorded a minimum temperature of 17.5°C in her study in the Loxahatchee River. Lower temperatures observed during my study may explain the absence of *Halosphaeria quadricomuta*, a typically common species.

Hurricanes and tropical storms can also create differences in species diversity. During the 2005 study by Kukich, Hurricane Wilma, a Category 3 storm, made landfall on the southwestern coast of Florida between Everglades City and Cape Romano on October 4, 2005. Maximum sustained winds over the Florida coastal everglades were estimated to be near 105 km h^{-1}, and for a time Wilma was the strongest Atlantic tropical storm on record with a minimum

central pressure at the time of peak intensity of 882 mb (Castaneda-Moya et al.). The large scale physical damage to mangrove forest structure included defoliation, tree snapping, and uprooting.

Castaneda-Moya *et al*. (2009) followed the passages of Hurricane Wilma across Shark River in the Florida coastal Everglades and quantified sediment deposition and nutrient inputs on the mangrove systems of the river. The bulk density, organic matter content, total nitrogen (N) and phosphorus (P) concentrations, and inorganic and organic phosphorous pools differed from surface mangrove soils at each site. Total phosphorus inputs from storm derived sediments were equal to twice the average surface soil nutrient nitrogen density creating a phosphorus gradient along the river moving west to east (Castaneda-Moya et al.). Lou *et al*. (2005) observed that nitrogen limitation favored the expression of ligninolytic enzyme systems in certain species of marine fungi. The dramatic change in soil nutrient composition may have limited the growth of more fastidious species and favored certain species with a more broad range of physiological requirements. During my study no hurricanes made landfall and the addition or turnover of nutrients experienced during Kukich's (2005) study did not occur.

4.2 Comparison of Species Composition along Henderson Creek

Kohlmeyer (1979) found *Lulworthia* sp. (20%), *Leptosphaeria australiensis* (15%) and *Phoma* sp. (10%) to be the most frequent species in mangrove environments (J. Kohlmeyer & Kohlmeyer, 1979). Hyde and Jones (1987) found *Halocyphina villosa* and *Lulworthia grandispora* to occur at a high frequency occurring species in the Seychelle Islands. Additionally, Jones *et al.* (2005) found *Kallichroma enalia, Leptosphaeria australiensis Lulworthia grandispora, and Verrculina enaila* to be the most frequently occurring species in the Bahama Islands. The aforementioned studies on the east coat of Florida by Mickel (2000), Vogel (2002), and Kukich (2005) had similar common species including *Antennospora*

quadricornuta, Cirrenalia macrocephala, Leptosphaeria peruviana, Payosphaeria minuta, Trichocladium alopallonella, and *T. lignincola.* While the dominant species observed in this study are similar to past reports from mangrove environments there are some notable differences. *Antennospora quadricornuta* (*Halosphaeria quadricornuta)* is common on Florida's East coast, but this species was not observed in this west Florida coast study. Adams (2003) showed that fungal colonization of mangrove substrates occurs at all times during the year by determining the relative amounts of ergosterol content in wood test panels and in mangrove wood. Erogosterol is a unique sterol found in the plasma membrane of fungal cells. Erogosterol levels were used to quantify the relative biomass of colonizing marine fungi and in turn patterns of fungal activity and colonization. Adams (2003) found a distinct seasonal pattern in ergosterol levels in each substrate tested. Overall concentrations increased during the late spring and summer months. This could provide insight to differences seen in species composition between my study sites and past studies. It is possible that certain time periods offered more favorable conditions for growth and reproduction by having more available substrata to colonize.

Colonization may occur throughout the year as Adams (2003) observed. However, species diversity and composition possibly differ when comparing studies as a result of varying substrate dimensions and condition as well as sample type (i.e branches, prop roots, leaves). *In situ* substrates submerged and colonized for a longer time period display less species diversity than freshly submerged test panels. Species diversity in my study for example was highest in the first and second test periods (4/1/2009-7/9/2009; 4/1/2009-10/15/2009) and supported the ergosterol data reported by Adams (2003). These time periods seemed to provide the most optimal conditions for growth and reproduction of a majority of species. Jones (2005) reported *Lulworthia* sp. as being one of the most frequently identified species in mangrove systems.

However, species of this genus occurred at a low frequency in this study. Jones (2005) sampled drift wood, mangrove wood, salt marsh plants, and seaweeds over a few days in the Port St. Lucie area on the east coast of Florida. It is difficult to compare long term seasonal studies such as the one presented here with short term collections which may provide an inaccurate view of species diversity and frequency of occurrence in a selected geographic area.

4.3 Geographic Distribution

Hughes (1974) reviewed the geographic distribution of higher marine fungi on a global basis and grouped them into four main categories reflecting their varying temperature requirements for growth and sporulation. These included cosmopolitan species, cold water species, temperate species, and warm water species (tropics and subtropics). Bebout *et al*. (1987) provided evidence suggesting that isotypes occur and these isotypes have very specific temperature requirements for optimum growth. Comparisons of the marine mycota of Tenerife, which lies in the temperate zone, supports the observation that the geographical distribution of some marine fungi is influenced by water temperature, while others are more limited by the distribution of their host plants (J. Kohlmeyer, 1977). Several species found in this study are considered cosmopolitan species and are not restricted to the tropical isotherms. For example, *Trichocladium alopallonellum, Zalerion maritima, Cumulospora maritima, Leptosphaeria australiensis,* and *Cirrenalia* species occur in temperate and tropical locations (Hughes, 1968). In this study, *L. australiensis* and *T. alopallonelum* were dominant at each site and throughout the year. The occurrence of some species however appears to be affected by ambient salinity. *Cirrenalia* species occurred most often at the high salinity site whereas some species of *Lulworthia* only occurred at the low salinity sites.

4.4 Species Dominance

A dominant species is a species of plant or animal that is particularly abundant or controls a major portion of the energy flow in a community. Dominant species can be viewed as those that prevent other species from becoming established on a common substrate possibly through antagonistic interaction. A functional example of this would be the phenomenon of competition. If a single species can outcompete another, it may in turn inhibit the growth and overall success of its competitors. When fungi begin to colonize a new substrate that is abundant in nutrients they typically undergo a period of rapid growth which inhibits the establishment of competitive species (Alexopoulos, C.W, & Blackwell, 1996). The most critical point in competition occurs when nutrients become limiting to growth. Species able to exploit the remaining resources typically have a competitive advantage over less adaptable species (Gow, G.D, & Gadd, 1999). Exerting antagonistic effects and ultimately suppressing the growth of other fungi was first reported for *Ceriosporopis halima* (J. D. Miller, 1986). Marine species commonly produce antifungal compounds whereas terrestrial species commonly produce secondary metabolites to inhibit competing bacteria. Metabolites can be used by fungi to expand their niche as well as to exclude other fungal species.

In a study conducted by Tan *et al.* (1995), three marine fungi (*Aigialus parvus, Lignincola laevis,* and *Verrculina enalia*) were grown both in pure and in mixed culture. All species were able to develop numerous ascomata when grown alone, but ascomata development was limited when these species were grown in combination. Growth of *L. laevis* was suppressed by *A. parvus* and/or *V. enalia*. It was discovered that *A. parvus* produced a number of bioactive compounds including hypothemycin (a known antibiotic), new macrolides, and a new ketene acetal (Tan, Teng, & Jones, 1995). Other marine species that produce known antifungal compounds include *Halocyphina villosa, Lignincola laevis, Zopfiella marina, Preussia*

aurantiaca, Dendryphiella salina, Phoma sp., *Leptosphaeria oraemaris,* and *Corollospora pulchella* (Pietra, 1997). Many of these species including *Halocyphina villosa* and *Phoma* sp. were collected in this study and were dominant or occurred in high frequencies at each site.

Antifungal compounds are commonly species specific. They are most useful ecologically when a species is initially colonizing a new substrate and nutrients are readily available. These compounds inhibit other species from becoming established on the substrate. *Halorosellnia oceanica* is known to produce potent antifungal compounds which inhibit fungal cell wall biosynthesis. In this study when *Halorosellinia oceanica* began to sporulate, it became the most visibly dominant fungus on each test panel. It frequently colonized entire sections of test panels possibly prohibiting other species from growing successfully. One could attribute the success of this species to the secretion of its antifungal compounds.

Not all secreted compounds serve as competitive inhibitors. For example *Pyrenographa xylographoides* which was isolated in pure culture from test panels in this study secretes a compound that causes the agar medium to become purple. This yet to be characterized compound was reported previously by (Alias, Hyde, & Jones, 1996). It is probably not an antifungal compound because, the secretion did not appear to restrict the growth of other fungal species on the wood test panels. Similarly, Strongman (1987) studied the interaction between *Lulworthia* sp., *Cirrenalia macrocephala,* and *Trichocladium alopallonella* and did not observe any interference behavior. This suggested that not all species secreted inhibitory chemicals or other antifungal compounds.

A typical way to outcompete other species for available subtrate is to outgrow them. In fungal communities, individual species referred to as necrotrophs may overgrow and digest the hyphae of less successful competitors. Burrnett (1969) showed that necrotrophs accomplish this

by producing enzymes capable of degrading the hyphal wall of the prey. In this study it was noted that *Periconia prolifica* displayed widespread growth on test panels when compared with other species. It did not however seem to chemically hinder other species' growth as was observed in *H. oceanica. Periconia prolifica* has not been shown to produce antifungal compounds or other inhibitory compounds. It is more likely that *P. prolifica* utilizes the "overgrowth" tactic to outcmpete other species on selected substrates. *Periconia prolifica* occurs over a broad range of salinities as shown in studies in Hong Kong, Goa (Virmoed et al 1982 and 1986), and India (Vishwakiran *et al 2001).*

Fungi can also participate in commensal relationships in biological communities. Tubaki (J. Kohlmeyer & Kohlmeyer, 1979) observed how *Corollaspora maritima* and *Ceriosporpsis halima* benefit one another. *C. martitima* has the ability to assimilate sodium nitrate unlike *C. halima.* When grown in a mixed culture *C. halima* used the ammonium created as a waste product by *C. maritima.* In my study *C. californica* and *C. halima* were observed together, with a maximum frequency of occurrence at the high salinity site. It is possible that these two species were serving to benefit each other in the same ecological niche.

4.5 Wood Decomposition Process

Many organisms in addition to fungi contribute to the biological deterioration of wood. While the exact contribution by each biological group is unclear, the chemical level of mycotic activity can be determined by measuring the levels of pentadeconoic and heptadeanoic fatty acids. Carbon (18) and carbon (22) are specific fatty acids indicative of fugal activity (J. D. Miller & Jones, 1985). Higher filamentous fungi will attempt to colonize most wood substrates for a certain period of time or until nutrients are exhausted (J. Kohlmeyer & Kohlmeyer, 1979). Lignin in the cell wall of wood however combines with neighboring polysaccharides to form

lignocellulose, making wood highly resistant to microbial activity. In order for any organism to utilize the cell wall polysaccharides, the lignocellulose must first be broken down to utilizable molecules (Mouzouras, 1986). Marine fungi possess a wide range of lignin modifying enzymes called cellulases. These allow fungi an advantage over species unable to utilize the cellulose in lignocellulose complexes (Pointing et al., 1998). In marine environments these enzymes must be stored within a membranous sheath produced by the fungus to prevent enzyme dilution by the ambient water (Mouzouras, 1989b). Microbial success on wood substrates can also be hindered by substrate density, chemical composition, thickness of cell walls, and the size of the cells (Gray, 1959).

Marine Ascomycetes and Deuteromycetes cause “soft rot” which refers to the condition of plant parts after they have become softened by the action of fungi and bacteria. Soft rot fungi break down a plant’s lignin by secreting laccase and peroxidase (Mouzouras, 1989a). Soft rot is restricted to the substrate’s surface layers as fungal hyphae can usually only penetrate a few millimeters below the surface layer due to restrictions imposed by their intrinsic high oxygen requirement (J. Kohlmeyer & Kohlmeyer, 1979). Fungal hyphae growth starts in the lumina of the cell. Hyphae pass through the dense S3 layer of the cell wall and grow into the less lignified S2 layer where they become oriented in the same spiral pattern as the cellulose microfibrils. The S2 layer of the cell wall contains a high concentration of energy rich cellulose which serves as a primary energy source. Basidiomycetes in comparison cause “white rot” where the secretion of cellulolytic enzymes forms erosion troughs and thins the cell walls. These enzymes easily diffuse from the hyphae into the substrate (Leightley, 1980). In comparison white-rot fungi are capable of decomposing polysaccharide and lignin whereas soft-rot fungi decay cellulose and hemicelluloses (Luo, Vrijmoed, & Jones, 2005). Soft rot fungi are more restricted and exhibit a

more controlled process of hyphae growth within the wood's cells. Soft rot fungi are considered to be more related to white rot fungi due to a sheath that surround the hyphae and thus restricts the movement of deteriorating enzymes.

Pointing *et a.l* (1998) studied lignocellulose degrading enzyme activity in fifteen marine fungal isolates. The isolates were tested for their ability to produce cellulose and lignin modifying enzymes. *Torpedospora radiata* and *Nia vibrissa* displayed high cellulase activity which correlated with their ability to grow well on cellulose materials. In contrast *Tromatosphaeria striatispora* and *Helicascus kanaloans* displayed significantly lower cellulase activity which explained their slow rate of vegetative growth. In my study *H. kanaloanus* had a low frequency of occurrence (4.2%) and only occurred at the moderate salinity site which may be explained by its lower cellulase activity as reported by Pointing *et al.* (1998) and its inability to digest lignin. Cellulose degrading enzymes while common among marine fungi have a range of physiological optima in respect to primary carbon sources, pH, and salinity requirements (Pointing et al., 1998). Lou *et al*. (2005) studied twenty-nine fungal isolates from tropical and subtropical mangrove/marine habitats and screened them for the presence of lignocellulose-degrading enzyme activity in agar media. Lou *et al.* (2005) found that the most common enzyme produced was endoglucanase and that 72% of the fungi produced a combination of endoglucanase, xylanase, and laccase. Lou *et al*. (2005) also found that the soft-rot fungi are weak in ligninolysis and that their laccases are too low to oxidize the recalcitrant non-phenolic moieties in lignin. Fungi possessing only one of the three lignocellulose degrading enzymes were not be as capable of decomposing wood substrates as those possessing all three. Some of the less competitive species identified in my study may not be fully able to synthesize the enzymatic combinations to be successful which was also suggested in previous studies of marine fungi

(Sutherland, D.L, & Speedie, 1982). A species' particular ability to produce a single or combination of enzymes could explain different species compositions seen in aforementioned studies as well as the prevalence of certain species in my study. Rohrmann *et al*. (1992) tested for the presence of lignocellulose-degrading enzymes in marine fungal isolates and ranked the tested species by their ability to produce either single or multiple lignocellulolytic enzymes in addition to the efficacy of the enzymes. He found that *Amylocarpus encephaloides* and *Ceriosporopsis halima* exhibited high levels of peroxidase activity and low cellulasese activity. In my study these two species were observed most often at the high salinity sites which correlate well with the finding that peroxidases are more active in high salinity environments whereas cellulases are inhibited. In comparison it was also discovered that *Lulworthia lindroidea, Cirrenalia tropicalis,* and *Varicosporina ramulosa* had higher levels of cellulases and low levels of peroxidases. These three species only occurred at the low salinity environments in my study.

Woodborers such as shipworms also help to promote fungal growth on woody substrates. The tunnels these organisms create provide additional surface area and substrates for fungi to colonize (J. Kohlmeyer & Kohlmeyer, 1979). These tunnels however, can be anoxic and thus serve as hostile areas for fungal colonization. Certain fungi including *Cirrenalia macrocephala* do however, thrive in these tunnels. *C. macrocephala* produces abundant conidia when growing in the abandoned tunnels of isopods such as *Limnoria* and *Sphaeroma* species (J. Kohlmeyer & Kohlmeyer, 1979). Soft-rot decay requires an exceptional amount of oxygen to be present, restricting the fungus to the upper surfaces of the substrate. By utilizing the respiratory pits created by the woodborer the fungal hyphae are allowed entry into the interior of the wood.

The rate of wood decomposition in marine environments is also affected by ambient salinity levels. In Eaton's (1976) cooling tower study, the rate of wood deterioration was directly

proportional to the salinity of the water flowing through the cooling tower. This trend was also noted in this study. While all panels exhibited some degree of deterioration after 12 months, the panels at the highest salinity site had significantly more deterioration. The wood at the high salinity site had increased fouling and in turn more damage by woodborers and barnacles (*Cirripeia* sp.). The extensive fouling and increased salinity led to the drastic decomposition of these test panels relative to those in the lower saline sites.

During the fourth period of my study (4/1/2009-4/7/2010) test panels at the high salinity site had the most extensive fouling and commonly fell apart during extraction from the water. This revealed extensive tunneling in the wood test blocks. At this site several species including *Cirrenalia basiminuta* had its highest frequency of occurrence (87.5%). The species richness also reached a maximum of fifty-four species. The tunnels may have created a additional surface area allowing more species to colonize the substrate and increased the oxygen content allowing soft-rot fungi to penetrate deeper into the substrate and access additional nutrients.

4.6 Host specificity and Substrate Preference

Hyde (1986) suggested that most marine fungi occurring on mangroves are substrate specific rather then host specific. Hyde cited the marine Basidiomyte *Halocryphina villosa* as it was only found on submerged mangrove wood samples. Host specificity has been addressed by Hyde and Jones (1988), Hyde (1990a), and Hyde and Lee (1995). In each study it was concluded that in mangrove habitats there is little evidence for host specificity. There are exceptions however. One example is *Didymosphaeria rhizophore* which is found exclusively on the red mangrove *Rhizophora mangle* (J. Kohlmeyer & Kohlmeyer, 1979).

In this study a single side t-test determining the significance of fungi colonizing Pine (softwood) or Oak (hardwood) produced a p-value of 0.1534 (Table 7). This signifies that the

fungus in this study did not exhibit a substrate preference. In most cases many fungi grew on both substrates at some time during the study. Peterson and Koch (1997) determined the substrate preference of lignincolous marine fungi on mooring posts of Oak (*Quercus* sp.) and Larch (*Larix* sp.) in Svanemollen Harbor, Denmark. They found nearly twice as many fungal species on Oak compared with Larch wood. They concluded that hardwoods are a more favorable substrate for growth rather than soft woods which tend to leach nutrients more quickly. In contrast Johnson (J. Kohlmeyer & Kohlmeyer, 1979) found more fruiting bodies on soft wood compared with hard wood. Adams (2003) quantified the amount of ergosterol on submerged pine and oak wood as a measure of fungal biomass. Adams (2003) found that the hard woods had low initial amounts of erogosterol, but over time the amounts increased and remained stable. Miller *et al*. (1985) also saw this pattern in ergosterol content over time for submerged wood substrates. It was concluded that the process of colonization of hardwood is slower than that of softwoods but the more stable substrate allows for a more diverse fungal community. In my study the soft wood had the highest total number of species during the first test period (97) after which the total number decreased in each test period. This may be due to the more rapid leeching of nutrients from the softwood which may have resulted in less available nutrients by the fourth test period (4/1/2009-4/7/2010). The hard wood in comparison showed no discernible trend in species number. After the first test period the number of total species was lower but more stable when compared with the submerged pinewood test panels (between 86 and 89). These findings corroborate those of Adams (2003) and may explain why there was little change in total species numbers on the hardwood test panels over time.

Peterson and Koch (1997) found *Trichocladium achrasporum* only on softwood substrates. In my study however, *T. achrasporum* was found on soft and hardwood test panels.

The ability of fungi to colonize wood in marine environments may not be a result of preference, but rather the lignocellulolytic decomposing enzymes the fungus possesses. It is possible that in different ambient conditions these enzymes may be less or more active as parameters such as pH, temperature, and salinity alter the enzyme's activity. Laccases for example from terrestrial fungi are inhibited by halides. Physiological studies of *Phanerochaete chrysosporium* also demonstrated that nitrogen limitation favored the expression of lignocellulolytic enzyme systems in certain species of marine fungi (Luo et al., 2005).

Species found less frequently on one type of wood may lack the proper combination of enzymes to degrade and colonize the surface. Lou *et al.* (2005) found that soft-rot fungi are weak in ligninolysis. Enzymological studies revealed that the redox potentials of fungal laccasses range from 0.4 to 0.8V which is much lower than those of ligninolytic peroxidases. Laccase is thus considered ineffective alone to oxidize non-phenolic moieties in lignin which comprise up to 90% of the polymer. Poor lignin-degrading capabilities of certain marine fungi could explain the absence of certain fungi such as *Halosphaeria quadricomuta* in my study. *Halosphaeria quadricomuta* was collected in studies on the east coast of Florida (Adams, 2003b; Kukich, 2005; Vogel et al., 2008). This may also explain why certain fungi were only identified from one type of substrate. *Acrocordiopsis patilii, Allescheriella bathygena, Argentinomyces naviculisporus, Botryophialophora marina, Carbosphaerella leptosphaerioides, Carbosphaerella pleosporoides, Halosphaeria trullifera, Sphaerulina orae-maris,* and *Varicosporina ramulosa* only occurred on the softwood test panels. In comparison *Alternaria. sp., Arthrobotrys arthrobotryoides, Asteromyces cruciatus Digitatispora marina, Halonectria milfordensis, Lautospora gigantea, Lulwoana uniseptata, Nais inornata, Quintaria lignatilis,*

Rhabdospora avicenniae Rhizophila marina, Rostrupiella danica, Trematosphaeria mangrovei, and *Verrucaria allantoidea* only occurred on the hardwood test panels.

4.7 Species Richness and Biodiversity

The high number of filamentous fungi (116) identified in my collection at Rookery Bay underlines the rich diversity of marine fungal species typically found in mangrove environments. Lignocellulose substrata support the greatest diversity of fungal species when compared to algae, sea grasses, and angiospems (K. D. Hyde & Jones, 1989c). Species diversity is typically greatest during the early period of substrate colonization (Alexopoulos et al., 1996). At that time there is usually no single dominant species whereas once a fungal community is established it becomes difficult for other species to colonize the area. This effect is commonly referred to as the prior colonization effect (Dix 1964 in Dix and Webster 1995). The community structure itself changes over time through species succession and is linked to several ecological factors including levels of nitrogen, carbon, water, and access to nutrients (Dix & Webster, 1995). Fungi that are able to overcome the physical and chemical barriers presented by wood substrata have an initial competitive advantage over other species. As a fungal community matures species diversity may decrease and one or two species may establish dominance. Dix and Webster (1995) studied French bean roots (*Phaseolus vulgaris)* and observed that exposed roots were initially colonized by 15-19 species of fungi, where after the community matured the overall diversity decreased to 6 species. After all the available niches are filled the arrival and establishment of a new species would have to be facilitated by the departure of an all ready established species (Wildman, 1992).

In a study of mangrove communities in south Florida Newell (1976) defined four stages of succession. In stages 1 and 2 phylloplane fungi and fungi of the mitosporic taxa are dominant.

In stages 3 and 4 these fungi become less dominant and are replaced by facultative marine species such as *Cytospora rhizophore, Lulworthia* sp., *Periconia prolifica, Robillarda rhizophore,* and *Zalerion varium* (Newell, 1976).

In my study a pattern of species dominance was observed on all wood test panels. On most panels *Halorosellinia oceanica* was not seen in the early stages. After three to four weeks however, *H. oceanica* became dominant on every test panel. After seven weeks it became exceedingly hard to find other new species. This was most likely due to the utilization of nutrients and secretion of inhibitory compounds by *Halorosellinia oceanica.*

The species richness found in our study follows the trend described by Dix and Webster (1995). The species richness, percent species, and Shannon-Weiner Diversity index are all highest during the first test period (4/1/2009-7/9/2009). As time progressed each component decreased as Dix and Webster (1995) predicted. Test panels at the site of moderate salinity were the only ones to exhibit the expected decline in total number of species over the 1-year testing period. Species number on the wood test panels at the high salinity site initially decreased as expected but then the species number increased to a total of fifty-four species in the final period (4/1/2009-4/72010) (Table 6). During this increase both the temperature and salinity were higher relative to the other study sites. Ritchie (1957) describes this combination as ideal for increased species diversity which may explain the higher species diversity that occurred in the last period (4/1/2009-4/72010). Total number of species on test panels at the low salinity site fluctuated through the duration of the study, but did reach a minimum in final period as predicted by Dix and Webster (1995).

4.8 Frequency of Occurrence

Prior to the 1980's there was little information on the frequency of occurrence of marine fungi on a particular substrate or their role in the degradation of organic matter in mangrove ecosystems (Venkateswara Sarma, Hyde, & Vittal, 2001). Early studies on fungi colonizing mangroves were taxonomic and focused on cataloguing and describing new fungal taxa from specific areas (Cribb & Cribb, 1955; J Kohlmeyer & Schatz, 1985). Recently there have been more ecological studies of manglicolous fungi providing information on the frequency of species occurrence (Alias, Kuthubutheen, & Jones, 1995). There is a paucity of data on the frequency of occurrence of manglicolous fungi in the western Atlantic Ocean. Most studies use the sporulation of fungi on a given substratum to determine fungal diversity. Bioactive compounds and antifungal chemicals produced by individual species however can alter the observed from the actual sporulation and diversity totals. The percentage of occurrence is an expression of the frequency of collections of fungi and provides a reference of the more common fungi in a given area (K. D. Hyde & Jones, 1988). The frequency of occurrence is based on the percentage of occurrence of fungi and these are subsequently categorized into frequency groupings (e.g >20%= very frequent; or <20%= less frequent) (Sarma & Hyde, 2001). Leong et al 1991 classified fungi as being very frequent (>20%), frequent (10-20%), and infrequent (<10%). In this study fungi are classified as very frequent (>80%), frequent (<70-60%), rare (<50%).

The Ascomycota comprised the most abundant group observed in this study. The Ascomycota and Deutermycota included several species that only occurred once throughout the entire study. Venkateswara Sarma *et al.* (2001) reported on the frequency of occurrence and biodiversity of fungi from mangroves on the east coast of India. The fungi with the highest frequency of occurrence included *Verruculina enalia, Rhizophora apiculata, R. marina,* and *Dactylospora haliotrepha*. Mickel (2000) found that *Halosphaeria quadricornuta* had the

highest frequency of occurrence in his study on fungal distribution in The New River in Broward County, Florida.

Halosphaeria quadricornuta is a common marine species but was not seen at any site in this study. Little information is available on the effect of temperature on *H. quadricornuta*. Kohmeyer (1968) reported that spores fail to germinate below 20°C and cultures produce no growth below 28°C. Distribution of this species is thought to be temperature dependant (Booth, 1983). During the third test period in my study (4/1/2009-1/7/2010) there was a noticeable temperature drop from 35°C to 13°C. *Halosphaeria quadricornuta* may not have been able to sporulate because of the lower temperature as described by Booth (1983). *Verruculina enalia* was also collected in my study, however it had a lower frequency (25%) in comparison to the collection by Venkateswara Sarma *et al.* (2001). This also may have been a result of the ambient water temperature decline.

Frequency of occurrence is altered by environmental parameters such as salinity and temperature. At each site certain species frequencies were correlated to the salinity gradient present in Henderson Creek. *Amylocarpus encephaloides, Lindra hawaiiensis, Acremonium tubakii, Amorosia littoralis,* and *Cirrenalia pseudomacrocephala* all had frequencies of occurrence that increased as the salinity increased along the salinity gradient, reaching a maximum frequency of occurrence at the high salinity site (Table 1). In contrast *Payliomyces lentifer, Cumulospora marina, C. varia, Trichcocladium melhae,* and *Zalerion maritima* all progressively decreased from the high to low salinity site and had a maximum frequency of occurrence at the low salinity site (Table I). The temperature at all three sites was relatively constant suggesting that the observed trends in the frequencies may be attributed to changes in salinity. Few reports are available for the effect of salinity on tropical fungi. Hyde (1992)

suggested that the distribution and frequency of marine fungi is limited by periods of higher salinity and therefore the mycota was likely to be similar throughout that salinity range of mangroves. In my study the mycota at each station was similar and the patterns of increasing/decreasing frequency in correlation to the salinity are consistent with Hyde (1992) suggesting that frequencies are affected by and possibly limited by salinity changes. Some species however, showed consistent frequencies at each station such as *Lignincola laevis* and *Alternaria* sp. which demonstrates that salinity does not alter the frequency of all marine species. Further studies investigating the effect of salinity on frequency and seasonal succession patterns might further the role played by by salinity and temperature.

Species frequency may also be affected by the substrata examined. Some fungi are known to occur more frequently on particular areas of trees or bark. Reported species frequency can therefore be a reflection of the number of samples collected as well as the substrate sampled. Ravikumar and Vittal (1996) reported on the fungi colonizing *Rhizophora apiculata* and *Rhizophoa mucronata* off the east coast of India. It was concluded that different areas of the same host plant are colonized by different fungal species. The greatest diversity and the highest total number of species of manglicolous fungi were found on the prop roots

In my study fungi were not restricted to one area of the test block, and frequently colonized all available space. The study site however was lined with red mangrove trees (*Rhizophora mangle).* We were unable to sample the prop roots and other parts of these trees as they were not accessible without the use of a boat. A future collection of marine fungi from the mangroves along the river compared to the findings of this study may reveal host specific fungi not seen in this study. A comparative study may also reveal if the species composition on the

mangroves is similar in regards to the high species diversity seen on wood test blocks in this study.

4.9 Seasonal Occurrence

Available information on seasonal occurrence of manglicolous fungi is sparse. In Sierra Leone, Aleem (1980) observed that mangrove fungi exhibited a seasonal periodicity with greater species diversity and fungal growth in the wet season (May-November). Species including *Haligena viscidula, Leptosphaeria australiensis, L. avicenniae, Rosellina* sp., and *Torpedospora radiate* occurred more frequently towards the end of the rainy season (September-October). Sarma and Vittal (2001) saw similar trends on the east coast of India. Aleem (1980) suggested that certain species of fungi may sporulate more frequently during the rainy season and others during the dry season. In my study it was thus hypothesized that there would be a difference in species composition and/or total number of fungi over a one year time period. However, we were not able to establish a distinct pattern of seasonality in the occurrence of fungal species. Rookery Bay is a part of the subtropical zone as defined by Hughes (1974). Water temperature is typically constant and a distinct pattern of seasonality apparently does not occur in this type of subtropical location.

4.10 Effect of Temperature

Booth and Kenkel (1986) suggested that ambient water temperature is the single most important factor in determining the geographical distribution of marine fungi. Byrne and Jones (1974) demonstrated the effect of temperature on the marine basidiomycete *Digitatispora marina* on test blocks of *Fagus sylvatica* in the Langstone harbour in Portsmouth, England. When the temperature dropped below 10°C *D. marina* sporulated on the wood blocks, but once the temperature reached over 10°C the fungus stopped fruiting. Panebianco (1994) examined the

temperature dependant growth of nineteen temperate, tropical, and cosmopolitan species of marine fungi at 10, 20, and 30°C. His study showed that cosmopolitan species grew at all temperatures and exhibited no preference for optimum growth (Panebianco, 1994). Tropical species in comparison showed optimum growth rates at 30°C and no growth at temperatures less than 10°C. Temperate species showed a similar temperature preference, but their optimum growth was at 20°C for most species, except *Marinospora calyptrate* and *Amylocarpus encephaloides* exhibited growth optimums at 30°C.

There is additional evidence that suggest that certain cosmopolitan species such as *Corollospora maritima* are capable of devloping distinct geographical isotypes based on their physiological responses to varying temperatures (Bebout, Schatz, Kohlmeyer, & Hailbach, 1987). Roberts et al (1995) conducted gene sequencing of the 18s gene in several isolates of *C. maritima* and found that the tropical isolates all grouped together. Two of the isolates from the subtropical collection formed their own unique group, and one isolate from Aldabra separated from both groups (Roberts, Mitchell, Moss, & Jones, 1995).

Palmero Llamas (2008) studied the effect of temperature and osmotic potential on the growth of *Fusarium solani*. He concluded that when grown at 25°C *F. solani* exhibited an optimum rate of growth and internal osmotic pressure. Isolates grown in lower temperatures displayed lower growth rates and lower internal osmotic pressure. Fungi maintain a high internal hydrostatic pressure (turgor) during cellular growth (Lew, 2010). If the osmotic pressure in the cell is less than the outside environment less water is taken up by the cell resulting in reduced growth. This may explain why sporulating fungi were less evident during the initial examination of the test blocks. After the test blocks were incubated at room temperature fungi were possibly more able to sporulate because the osmotic pressure increased relative to the ambient

extracellular environment. Prasannarai and Sirdhar (1997) also observed that the percentage and frequency of occurrence in fungi changed in relation to incubation times. They found that 70% of the total fungi encountered were seen in the first 6 months of incubation over an 18 month observation period. The incubation process may have favored certain mitosporic fungi and not reflect the totality of the colonizing species. Changes in osmotic potential may also account for the lower species diversity observed in the third and fourth testing periods (4/1/2009-4/7/2010). During those periods the temperature reached a minimum which would have lowered the osmotic pressure in less resilient species as described by Lew (2010). Species including *Acrocordiopsis patilii,Amarenomyces ammophilae, Capillataspora corticola, Caryospora australiensis, Dactylospora mangrovei, Digitatispora marina, Halenospora varia, Lignincola laevis, Macrophoma* sp., *Manglicola guatemalensis, Phaeosphaeria halima, Phomatospora nypicola, Quintaria lignatilis,* and *Ulocladium atrum* were only present in the first and second testing periods (4/1/2009-7/9/2009; 4/1/2009-10/15/2009) when the ambient water temperatures were highest.

4.11 Effect of Salinity

The identification of three distinct salnity zones along Henderson Creek presented an ideal location for a study of seasonal distribution of higher filamentous fungi along a salinity gradient. Downstream of Henderson Creek an inlet allows for the diurnal exchange of water and other materials. Lee and Yokel (1973) showed that the tides at Rookery Bay are a mix of diurnal and semidiurnal changes, with two high and two low tides per day of unequal amplitude during spring tides and two high and two low tides per day of nearly equal amplitudes during neap tide. The annual rainfall patterns were categorized by Shirley et al. (2003) (1) early dry (December through February), (2) late dry (March through May), (3) early wet (June through August) and

(4) late wet (September through November). Seasonal salinity changes within Rookery Bay occur in response to seasonal variations of total freshwater inflow (Shirley, O'Donnell, McGee, & Jones, 2003). Typically the highest salinity occurs during the late dry season and the lowest salinity in late wet season. In Shearer's (1972) study along the Paxtunt River in Maryland the ratio of Ascomycotina to Deuteromycotina increased as the salinity increased. Individual stations did not show an increased ratio as described by Shearar (1972), however the annual total ratio of Ascomycotina to Deutermycotina between stations did. The low salinity Visitor Center site had the lowest ratio, followed by the moderate salinity site, with the highest ratio occurring at the high salinity Field Station site.

High salinities have also been reported to reduce the production of cellulases and increase the function of peroxidases that various fungi employ to aid in the colonization of wood substrata (Pointing et al., 1998). Lou *et al.* (2005) tested for enzymatic activity and found 18 of 21 (ca. 86%) marine Ascomycete species were positive for laccase activity and Raghukumar *et al*. (1994) found 11 of 17 (65%) species produced laccases. Peroxidases have been more commonly reported from a variety of white-rot Basidiomycota (Hatakka, 1994). Lou *et al*. (2005) also quantified the effficacy of various lignocellulolytic enzymes in a number of marine fungal species. *Cirrenalia tropicalis* displayed above average secretion of cellulytic enzymes. In my study, *Cirrenalia tropicalis* displayed the lowest frequency of occurrence at the high salinity site. This may be attributed to the lower activity of its cellulytic enzymes at high salinities. The Basidiomycota in my study also only occurred at the moderate and high salinity sites. Increased activity of peroxidases in high salinity environments as reported by Pointing *et al*. (1998) may explain their absence from the low salinity site.

The third test period (4/1/2009-1/7/2009) was a period of instability as shown by a temperature decrease of the ambient water temperature by approximately 10°C and an increase in salinity of approximately 10ppt at each site. This period, in addition to the fourth test period (4/1/2009-4/7/2010), displayed the lowest total species richness and diversity. Eaton (1976) and Shearer (1972) pointed out that the Deutermycotina dominate in times of unstable conditions due to their less stringent requiremnts for reproduction. Ascomycotina in turn are more dominant during stable periods. The predicted high occurrence of the Deutermycota only occurred at the high salinity Field Station site. The Ascomycotina increased and the Deutermycotina decreased at the moderate and low salinity sites. This unexpected trend could be explained by the relative salinity levels at moderate and low salinity sites. The '*Phoma'* pattern describes a relationship between temperature and salinity and their effect on fungal sporulation and growth (Ritchie, 1957). Typically, fungal species grow best when salinity and temperature are correlated. Two examples are *Dendryphiella salina* and *Zalerion maritimum* which exhibit an increase in their salinity optimum for growth with elevation in temperature. Periods of high salinity and high temperature or low salinity and low temperature support the best growth. Periods of high and low temperatures or salinity yield low growth and development.

The salinity change at the low and moderate salinity sites may have still been at levels to support optimal growth. The high salinity and low temperature at the high salinity Field Station site may have been too unstable of an environment and detrimental for growth and reproduction

4.12 Adaptations – appendages

Many marine Ascomycetes found in mangrove ecosystems produce ascospores with appendages which aids in attachment by increasing the surface area thereby allowing for a higher chance of contacting a new substrate. Ascospore appendages decrease sedimentation rates, facilitate spore

flotation and aid dispersal (K. D. Hyde & Jones, 1989b). The appendages are typically rigid, chitinous or composed of gelatinous mucosaccharides (Jones & Moss, 1978). Ten different spore appendage types have been described in lignicolous marine fungi by Rees and Jones (1984) as well as Hyde and Jones (1989). Forms include: release of a drop of mucilage (*Lulworthia* species), cap-like appendages that uncoil to form long filaments (*Halosarpheia* species), sticky gelatinous sheaths (*Massarina* species), disk-like attachments, sticky vermiculate appendages that surround the spore (*Carbosphaerealla* species), ribbon-like appendages (*Corolllospora* species), tufts of fibrous appendages, irregular appendages, an adhesive spore wall, and several combination of the above. The muccosacharide appendages are thought to be the most useful since the spore can more easily adhere to a substrate (K. D. Hyde & Jones, 1989a; Rees & Jones, 1984)

The appendages of some of the ascospores observed in this study can be categorized into three groups: ribbon like appendages (*Corolllospora* species*)*, flexible filamentous appendages (*Ceriosporopsis* species), cap-like appendages that uncoil to form long filaments (*Halosarpheia* species) disk like appendages at the apices of the appendage (*Lulworthia* species, *Carbosphaerealla* species), and sticky gelatinous sheaths (*Massarina* species). Interestingly all of these species had low frequencies of occurrence (<50%) and were categorized as rare in my study. Mickle (2000) found *Lulworthia* spp. to occur frequently while both Adams (2002) and Kukich (2005) categorized it as being rare (<10% frequency of occurrence). This may suggest that appendages in marine fungi while helpful may not necessarily increase the rate of successful colonization. Additionally none of the dominant species in my study such as *Biflua physasca*, *Halorosellinia oceanica*, *Leptosphaeria australiensis* have appendages. This observation may suggest that dominant species are not necessarily aided by appendages. Future work to test the

percent of successful colonization of marine fungi with appendages relative to those without may lead to insight on why appendaged marine fungi do not have as high frequencies of occurrence.

5. Conclusion

In previous studies, changes fungal distribution attributed to fluctuations in salinity (Shearer, 1972), temperature (Kirk and Brandt, 1980), and dissolved oxygen concentrations (Kirk and Schatz, 1980).

While some of the species collected in my study are cosmopolitan and did not appear to be affected by the range of salinities others were affected by the extremes in the range of salinities found along the gradient. Numerous fungi including *Acrocordiopsis patilii* was restricted to the high salinity site while others including *Arthrobotrys arthrobotryoides* were only collected from the low salinity site. Other species demonstrated correlations between salinity and frequency of occurrence such as *Paraliomyces lentifer*. The high species diversity observed included a core group of dominant marine fungal species. Factors contributing to the success of dominant species include the production of various antifungal compounds and the ability to produce multiple lignin degrading enzymes. The study of interference behavior of marine fungi is of great importance to the discovery of new pharmaceutical compounds. The ratio of total number of Ascomyetes to imperfect fungi was also correlated to the salinity gradient along Henderson Creek. Salinity appeared to have more of an effect on the composition of and distribution of fungi. The higher species diversity seen in this study may be attributed to the nearby mangals surrounding each site and the infrequent occurrence of hurricanes during this study.

Temperature did not appear to alter species composition but it did lower the total species number at each site. No pattern of seasonal distribution was established along Henderson Creek in this study.

Few organisms are able to digest cellulolytic materials found in estuarine environments. The ability to breakdown and recycle cellulose therefore indicates that fungi are important contributors to the dissolved organic matter in estuarine and near-shore systems (D. Hyde et al., 1998). Most studies examine the taxonomy and geographical distribution of marine fungi, few however examine the occurrence and seasonal composition. More studies of the physiological behavior of marine fungi need to be conducted to better understand the importance and role of marine fungi in marine ecosystems.

6. Glossary

Algicolous fungi: Fungi growing on (or in) algae.

Aquatic fungi: Marine or freshwater fungi.

Ascocarp: Fruiting body of an Ascomycete, producing asci with ascospores.

Ascomycotina: Fungi that reproduces sexually in the phylum Dikaryomycota.

Ascus: The saclike reproductive cell of the Ascomycetes; the formation of usually eight ascospores is preceded in the young ascus by karyogmay and meiosis.

Ascospore: A spore formed in the ascus.

Basidiocarp: Fruiting body of a Basidiomycete, producing basidia with basidiospores.

Basidiospore: A spore formed on the basidium.

Carbonaceous: Charcoal-like, easily broken, dark-colored.

Cellulolytic: Cellulose decomposing.

Conidium: An asexual reproductive unit of the Deuteromycotina (imperfect fungi).

Conidiophore: A hypha bearing conidiogenous cell, which procude conidia.

Deuteromycota: Fungi that only reproduce asexually in the phylum Dikaryiomycota.

Germ pore: A thin area in the wall of a spore through which a germ tube may come out.

Hypha: A filament fungi, forming the mycelium.

Isothere: A line connecting points having the same mean summer temperature.

Lichen: A symbiosis between algae and fungi, usually resulting in an association that is morphologically different from both partners, usually producing specific lichen substances.

Lignocolous fungi: wood-inhabiting fungi.

Mangilcolous fungi: Living in mangroves.

Mycelium: A network of fungal filaments (hyphae).

Mycology: The science of dealing with fungi.

Mycobiont: The fungal partner in a lichen

Mycorrhiza: A symbiotic association between fungi and roots of higher plants.

Mycota: The fungal population of a particular area.

Phycobiont: The algal partner in a lichen.

Ramose: Branched.

Saprobe: An organism utilizing dead organic substrates as food.

Soft rot: Deterioration of wood by hyphae of higher fungi, which attack predominantly the unlignified layers of the cell walls, causing characteristic tunnels inside the walls.

Stroma: A mass of vegetative hyphae in, on, or under which fructifications are produced.

Verrucose: Having small warts or rounded processes.

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